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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Tezacaftor and Ivacaftor by RP-HPLC Method

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A B S T R A C T

The aim of present research work made to develop and validate simultaneous estimation of Tezacaftor and Ivacaftor was done by RP-HPLC. The optimized mobile phase was consists of Acetonitrile: Phosphate buffer pH 2.5 mixed in the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µ, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 274 nm. The flow rate was maintained at 0.8 ml/min. The linearity range of Tezacaftor and Ivacaftor were found to be from 25-125 µg/ml and the linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Tezacaftor and Ivacaftor LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Tezacaftor and Ivacaftor in formulation. So the method can be useful in the routine quality control of these drugs.

Keywords: Symmetry C18 column, Tezacaftor and Ivacaftor, RP-HPLC, Stationary phase, Mobile phase

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1. Introduction

Tezacaftor is a small molecule that can be used as a corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) gene function. It transport of charged ions across cell membranes is achieved through the cystic fibrosis transmembrane regulator protein. This protein serves as a channel and allows passage of charged ions such as chlorine or sodium. This process is also important for the movement of water in the tissues and for the creation of a thin mucus that can lubricate some of the organs and body tissues, including the lungs. In the F508 del mutation of

CFTR, one amino acid is deleted in the position 508 and thus the channel function is compromised and thick mucus is produced [5].

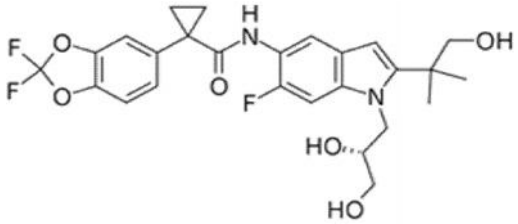

Fig 1: Chemical structure of Tezacaftor

Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of Cystic Fibrosis (CF) in patients aged 2 years and older. 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. CFTR is active in epithelial cells of organs such as of the lungs, pancreas, liver, digestive system, and reproductive tract. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes. As a result, CF patients produce a thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such as infections, lung damage, pancreatic insufficiency, and malnutrition[6].

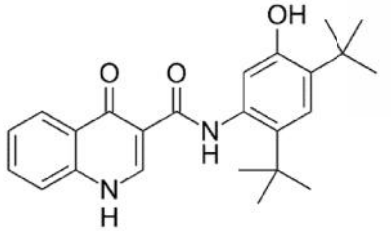

Fig 2: Chemical structure of Ivacaftor

Literature survey reveals that few methods have been developed for the simultaneous estimation of tezacaftor and Ivacaftor by HPLC[8,9,10]. The present attempt was made to develop simple, precise, accurate and robust RP-HPLC method for the simultaneous estimation of tezacaftor and ivacaftor in bulk and its formulation by changing of few chromatographic conditions.

2. Materials and Methods

Instruments used:

The following instruments are used to determination of tezacaftor and ivacaftor by RP-HPLC.

Table 1: List of Instruments

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A

Chemicals used:

The following chemicals are used to determination of tezacaftor and ivacaftor by RP-HPLC.

Table 2: List of Chemicals

S .No	Chemical	Brand
1	Tezacaftor	Symdeko
2	Ivacaftor	Symdeko
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCH)
5	Acetonitrile for HPLC	MOLYCHEM
6	HCl, H_2O_2, NaOH	MERCK

HPLC Method Development

Preparation of mobile phase:

Accurately measured 200 ml (20%) of above buffer and 800 ml of methanol HPLC (80%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. This was used as diluent.

Standard Solution Preparation:

Accurately weighed amount of 50mg Tezacaftor and 50 mg Ivacaftor were taken to a 100 ml clean and dry volumetric flask. This was then diluted with 70 ml of diluent and was sonicated. The volume was made to100 ml with the same solvent. This was taken as stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with the diluent to get final concentration of 75µg/ml.

Sample Solution Preparation:

Weight equivalent to 50 mg of Tezacaftor and Ivacaftor sample were weighed this was taken into a 100 ml clean dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and volume made up to the mark with the same solvent. This was taken as stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with diluent to get final concentration of 75µg/ml.

Wave length selection:

UV spectrum of 10 µg / ml Tezacaftor and Ivacaftor in diluents was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum, the wavelength of 274 nm was selected. At this wavelength Tezacaftor and Ivacaftor standards shows good absorbance.

Optimization of Column:

The method was performed with various columns like hypersil column, lichrosorb, and Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) was found to be ideal as it gave a good peak shape and resolution at 0.8ml/min flow.

Optimized chromatographic conditions

Mode of operation: Isocratic

Column **:** Symmetry C18 (4.6 x 150mm, 5µm,
Buffer pH **:** 2.5
Mobile phase **:** 20% buffer 80% acetonitrile
Flow rate **:** 0.8 ml per min
Wavelength **:** 274 nm
Temperature **:** ambient.

Run time **:** 7min.

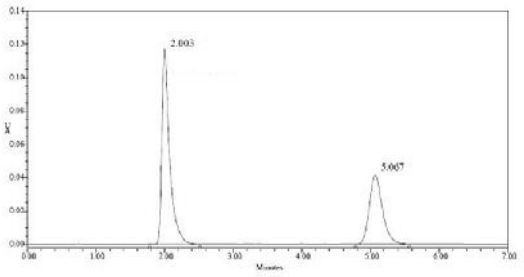

Fig 3: Chromatogram for Tezacaftor and Ivacaftor

Observation: From the above chromatogram it was observed that the tezacaftor and Ivacaftor peaks are well separated. Retention time of tezacaftor was 2.003 min and Ivacaftor was 5.067 min.

Method Validation

Method validation was done for the according ICH guidelines Q2(R1). The validation parameters like linearity, specificity, accuracy, precision, LOD & LOQ and robustness.

Linearity:

For determination of linearity five different concentrations i.e. 25%, 50%, 100%, 125%, 150% were prepared and injected in triplicate. Then plotting the graph concentration Vs peak area and measure the correlation coefficient. It was not more than 0.999.

Precision:

The standard and sample solutions of 75 µg/ml was injected five times in intraday and inter day, the peak areas were recorded .The mean and percentage relative standard deviation were calculated from the peak area.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same. Each solution was injected three times under optimized conditions and then calculate the mean percentage recovery.

LOD & LOQ:

The sensitivity of the proposed method for measurement of tezacaftor and ivacaftor were estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).The LOD and LOQ were calculated by using the slope and SD of response (intercept).The mean slope value and SD of response were obtained after plotting six calibration curves.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow: The sample was analyzed at 0.7 ml/min and 0.9 ml/min instead of 0.8 ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. phosphate buffer: acetonitrile was taken in the ratio 15: 85 v/v and 25:75 v/v instead of 20:80 v/v, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

3. Results and discussion

System Suitability:

The system suitability of the method was checked by injecting five different preparations of the tezacaftor and ivacaftor standard. The parameters of system suitability were checked. It was found from above data that all the system suitability parameters for developed method were within the limit. The results were shown in table 3.

Acceptance criteria:

- Resolution between two drugs should not be less than 2
- Theoretical plates should not be less than 2000
- Tailing factor should not be less than 0.9 and not more than 2.

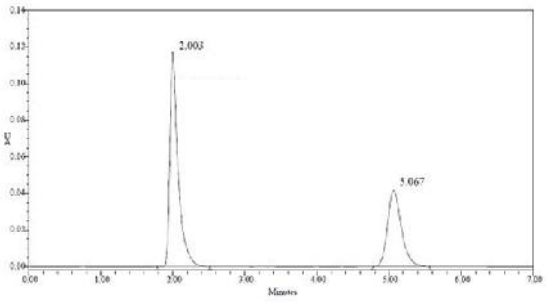

Fig 4: Chromatogram for system suitability

Linearity:

The linearity range was found to lie from 25% to 125%. The data was reported in table 4 & 5and fig 5 & 6.

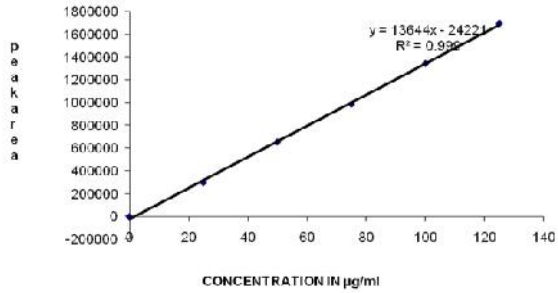

Fig 5: Calibration graph for tezacaftor at 274 nm

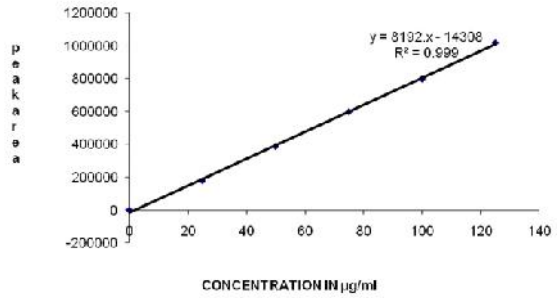

Fig 6: Calibration graph for Ivacaftor at 274 nm

Precision: Precision of the method was carried out for both sample and standard solutions as described under experimental work. The results were shown in table 5 & 6.

Accuracy: The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence the method is accurate. It was shown in table 8.

Robustness:

The standard and samples of Tezacaftor and Ivacaftor were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were shown in table 9 & 10.

Table 3: System Suitability results

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Tezacaftor	2.003	920101	116666	1.5	1.6	2711.8
2	Ivacaftor	5.067	552058	41531	11.0	1.3	3428.2

Table 4: Area of different concentration of Tezacaftor and Ivacaftor

Concentration (µg/ml)	Peak area of Tezacaftor	Peak area of Ivacaftor
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R2)	0.999	0.999

Table 5: Results of method precision for tezacaftor and Ivacaftor

Drug	Tezacaftor			Ivacaftor		
S. No	Sample area	Standard area	Percentage purity	Sample area	Standard area	Percentage purity
1	983375	971536	101.04	592403	577531	101.36
2	985049	973007	101.03	592352	580381	101.85
3	982956	975717	100.54	592357	577723	102.32
4	985219	978909	100.44	592323	582190	101.44
5	994145	981422	101.09	596525	583378	101.09
Average	983234	976311	100.84	592325	582755	101.24
%RSD	49.5	48.2	0.304	29.5	28.7	0.46

Table 6: Results of Intermediate precision for Tezacaftor and Ivacaftor

Drug	Tezacaftor			Ivacaftor		
S. No	Sample area	Standard area	Percentage purity	Sample area	Standard area	Percentage purity
1	979556	984395	99.30	583416	593403	99.12
2	982467	984039	99.64	583657	594352	99.01
3	979717	983976	99.36	584731	593357	99.52
4	978909	984278	99.28	583594	592673	99.61
5	981432	973915	100.57	597649	593671	99.12
Average	985321	984824	99.63	596537	592542	99.27
%RSD	48.2	48.5	0.54	29.3	29.2	0.27

Table 7: LOD & LOQ Results

Parameter	Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
LOD	Tezacaftor	56	176	3.14
	Ivacaftor	56	154	2.75
LOQ	Tezacaftor	56	563	10.05
	Ivacaftor	56	558	9.96

Table 7: Results of Accuracy

Spike level	Sample set no	Sample area		Assay		% Recovery	
		Tezacaftor	Ivacaftor	Tezacaftor	Ivacaftor	Tezacaftor	Ivacaftor
50%	1	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					99.7%	99.7%
100%	1	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					99.6%	99.8%
150%	1	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					99.7%	99.8%

Table 9: Results for effect of variation in flow

S. No	Peak area for Less flow (0.7 ml/min)		Peak area for More flow (0.9 ml/min)	
	Tezacaftor	Ivacaftor	Tezacaftor	Ivacaftor
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	986306	582223	976755	591667
%RSD	0.45	0.80	0.53	0.80

Table 10: Results for effect of variation in mobile phase composition

S. No	Peak area for Less organic (70%)		Peak area for More organic (90%)	
	Tezacaftor	Ivacaftor	Tezacaftor	Ivacaftor
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	987286	583658	986641	592877
%RSD	0.45	0.90	0.51	0.57

4. Conclusions

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Tezacaftor and Ivacaftor in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The silmultaneous estimation of Tezacaftor and Ivacaftor was done by RP-HPLC. The Phosphate buffer pH was 2.5 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µ, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 274 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Tezacaftor and Ivacaftor were found to be from 25-125 mg/ml. linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Tezacaftor and Ivacaftor LOD and LOQ was found to be within limit.

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