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A New Validated RP-HPLC Method for Simultaneous Estimation of Tezacaftor and Ivacaftor in Pharmaceutical Dosage Form

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ABSTRACT

A simple, selective, linear, precise and accurate reverse phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Tezacaftor and Ivacaftor in tablet dosage form. The chromatographic separation was achieved on Symmetry C18 4.6×150mm, 5µ column using a mobile phase consisting a mixture of Methanol: Water in the ratio of 65:35 v/v at a flow rate of 1ml/min at an ambient temperature and detection was carried out at 270 nm. The clear chromatography peaks were identified with retention times of 2.460 min for Tezacaftor and 4.312 min for ivacaftor. The proposed technique was validated according ICH guidelines in respect to specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The linearity was observed in the concentration range of 45-225 µg/ml for Tezacaftor and 10-50µg /ml for ivacaftor. Linear regression coefficient for both drugs was 0.999. The percentage recovery of Tezacaftor and ivacaftor was in between 98-102%.The %RSD for repeatability and intermediate precision was less than 2%. LOD was 0.83 and 1.3 and LOQ was 2.5 and 3.95 for Tezacaftor and ivacaftor respectively. The results of validation parameters were met ICH requirements. Hence, the proposed method can be used for the determination of Tezacaftor and ivacaftor in various pharmaceutical dosage forms during regular and quality-control analysis.

Keywords: Tezacaftor, Ivacaftor, Simultaneous estimation, RP-HPLC, tablets.

Introduction

Cystic fibrosis (CF) is a hereditary disease affects the endocrine, gastrointestinal reproductive, and respiratory systems. It causes the assemblage of abnormally thick mucus, leading to the obstruction. CF is caused by any one of several defects in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, such as F508del mutation, G551D mutation that causes the disease.[1] This life-restriction disease requires multiple daily medications to extend the life and get a better quality of life.

Many conventional regimens including pancreatic enzymesupplements,multivitamins, mucolytics, antibiotics,*bronchodilators*,and *anti*- inflammatory agents have been used for the treatment of CF. Tezacaftor (CFTR corrector) and Ivacaftor (Potentiator) are new drugs used in combination (brand name **Symdeko**) for the treatment of cystic fibrosis. Tezacaftor (LMF) is an aromatic amide, is a chemically 3- [6-[[1-(2, 2-difluoro-1, 3-benzodioxol-5-yl) cyclopropane carbonyl]amino]-3-methylpyridin-2-yl]benzoic

acid, Figure 1 with the molecular formula of $C_{24}H_{18}F_2N_2O_5$ and molecular weight is 452.414. It is a white to off-white powder that is practically insoluble in water (0.02 mg/mL). Tezacaftor acts as a chaperone during protein folding and increases the number of cystic fibrosis transmembrane conductance regulator proteins which are trafficked to the cell surface by targeting the defective F508del CFTR gene. [2] Ivacaftor (ICF) is an aromatic amide, chemically it is a N-(2,4-di tert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxamide, Figure 2 with a molecular formula of $C_{24}H_{28}N_2O_3$ and molecular weight is 392.499. Ivacaftor is a white to off-white powder that is virtually insoluble in water (<0.05 mg/mL). Ivacaftor is the first drug that treats the original cause rather than the symptoms of the disease. Ivacaftor is a potentiator of the CFTR protein a chloride channel present at the surface of epithelial cells in multiple organs, it increases chloride transport by potentiating the channel-open probability (or gating) of the G551D-CFTR protein. [3,4] Tezacaftor and Ivacaftor fixed-dose combination oral tablets are developed by Vertex Pharmaceuticals and both were approved by the FDA in 2015. [5] These drugs, when given in a fixed dose combination product rather than individual entities, has shown to get potential therapy in a condition of cystic fibrosis by correcting the defective protein. [6,7] Number of drugs are introducing into the market yearly. There is a time lag between the date of the prologue of a drug into the market and the date of its enclosure in pharmacopeias. Hence, standards and analytical methods either for the individual or combination of drugs may not be official in the pharmacopeias. Some analytical procedures are not accessible in the literature due to patent regulations. Analytical methods for the drugs in formulations are not available owing to the interference caused by the excipients. Therefore, it becomes essential to build up a newer analytical procedure for such drugs.

Literature survey reveals many analytical methods have been published for simultaneous estimation of Tezacaftor and Ivacaftor in bulk, pharmaceutical dosage forms and in biological samples. These methods are UV Spectrophotometric techniques, HPLC methods, UPLC method, stability indicating methods, and LC-MS/MS methods.[8-17]TheobjectiveofourstudyisthatHigh-performance liquid chromatography has an increasing growth in the analysis for the determination of API in various pharmaceutical formulations, which make it the most accepted and suitable technique for the determination. A successful attempt has been made for simultaneous estimation of LMF and ICF by RP-HPLC method in tablets. This research work was planned to develop a new, simple, accurate, reproducible, and economical method for simultaneous estimation of Tezacaftor and Ivacaftor in tablets by Reverse Phase High Performance Liquid Chromatography. The proposed method was validated in accordance to specificity, linearity, precision, accuracy, LOD, and LOQ, and robustness as requisite by the International Conference on Harmonization Q2 (R1) guidelines to support the suitability of the method.[18]

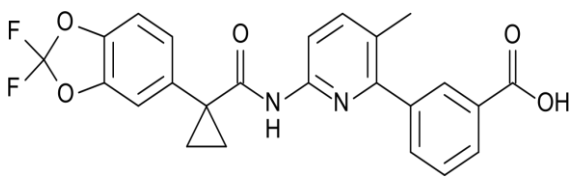

Figure 1: Structure of Tezacaftor

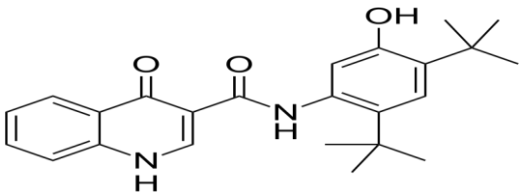

Figure 2: Structure of Ivacaftor

MATERIALS AND METHODS

Tezacaftor and Ivacaftor standard drugs were obtained as contribution samples from Aspen labs, Hyderabad, India. Methanol and water of HPLC grade were procured from E. Merk (India) Ltd. Worli, Mumbai, India. The 0.45µ nylon filters were purchased from Millipore. Tezacaftor (200mg) & Ivacaftor (125mg) tablets as **ORKAMBI** were obtained from Mukesh pharmacy,Hyd.

Instrumentation and chromatographic conditions

The HPLC system (waters) autosampler separation module 2695 consisted of high-pressure pump, PDA detector 996, and 10 µL capacity injector loops. The system was well equipped with empower 2 software for monitoring and processing of data. Other types of equipment like Sartorius digital weighing balance, Lab India pH meter, and Lab man digital ultra sonicator were used for sample and standard preparations. The analytical column used was Symmetry C184.6×150mm

Method development

Various mobile phases with different ratios were tested during the development of the RP-HPLC method suitable for the estimation of Tezacaftor and Ivacaftor in tablet formulation. These methanol and water in the ratio of 20:80, 60:40, 45:55, 70:30, 50:50, and 65:35 v/v. The mobile phase was selected for the sensitivity of the process, the time necessary for the analysis, easily available solvents, and simplicity of preparation. The mobile phase was premixed and filtered through a 0.45µm filter and sonicated for 10min to remove gases. Optimization of mobile phase was taken from various parameters such as retention time, number of theoretical plates, and resolution. The mobile phase was used as adiluent.

Preparation of standard stock solution of Tezacaftor

10 mg of Tezacaftor working standard was weighed and transferred into a 10ml of the clean dry volumetric flask; about 7ml of diluent was added, dissolved and then diluted to 10 ml.

Preparation of standard stock solution of Ivacaftor

10 mg of Ivacaftor working standard was weighed and transferred into a 10ml of the clean dry volumetric flask; about 7ml of diluent was added, dissolved and then diluted to 10 ml.

Preparation of working standard solutions of Tezacaftor andIvacaftor

135µg/ml of Tezacaftor and 30µg/ml of ivacaftor were prepared by diluting 1.35ml and 0.3ml of above stock solutions to 10ml with the diluent.

Preparation of mobile phase

650 ml of Methanol and 350 ml of Water were mixed together and the solution was degassed in digital ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum.

Preparation of sample solutions

Twenty tablets containing 200mg of Tezacaftor and 125 mg of Ivacaftor were weighed accurately and grounded to a fine powder, the powder which equals to each 10 mg of Tezacaftor and Ivacaftor drugs was precisely weighed and transferred into a 10ml of the clean, dry volumetric flask, dissolved completely in sufficient diluent, filtered the solution using 0.45-micron syringe filter, Sonicated for 5 min, and then diluted to 10 ml with diluent. 135µg/ml of Tezacaftor and 30µg/ml of ivacaftor were prepared by diluting 1.35 ml of Tezacaftor and 0.3ml of Ivacaftor to 10 ml withdiluent**.**

Procedure

A solvent system of Methanol and Water in the ratio of 65:35% v/v was found to be the most suitable mobile phase for ideal separation of Tezacaftor, and Ivacaftor. The mobile phase was pumped into the column at a flow rate of 1 ml/min. The column was set at ambient temperature and equilibrated with mobile phase for 30 minutes before the injection of solutions. 10µl of five replicates of both standard and sample solutions were injected into the chromatographic system and the areas of Tezacaftor and Ivacaftor peaks were measured. The detection of drugs was monitored at 270 nm. The runtime was set at 10 min. Under these optimized chromatographic conditions, the retention time for both the Tezacaftor and Ivacaftor was recorded from chromatograms. From the peaks of drugs, the % assay was calculated using the followingformula.

AS: Average peak area of standard preparation AT: peak area of assay preparation,
WS: standard weight of ivacaftor/ Tezacaftor in mg WT: Weight of sample in assay preparation
DT: Dilution of assay preparation DS: dilution of standard preparation P: purity of ivacaftor /Tezacaftor AV: average weight of tablets in mg
LC: labelled claim of ivacaftor/Tezacaftor

Method validation

Validation of the analytical method verifies that the individuality of the method if they persuade the requirements of the method. The developed method was validated for different analytical performance parameters such as specificity, linearity, accuracy, precision, LOD, LOQ and robustness according to ICH Q2 (R1) guidelines.

RESULTS AND SCUSSION

Study of retention time

A standard dilution of pure drugs having 135µg/ml of Tezacaftor and 30µg/ml of Ivacaftor were prepared in a diluent and loaded injection port of instrument fitted with a 10µl fixed loop. The solution was injected and chromatogram was recorded. The retention time of Tezacaftor was 2.456 min, and Ivacaftor was 4.312 min. The relevant chromatogram of standard solution is shown in Figure 3.

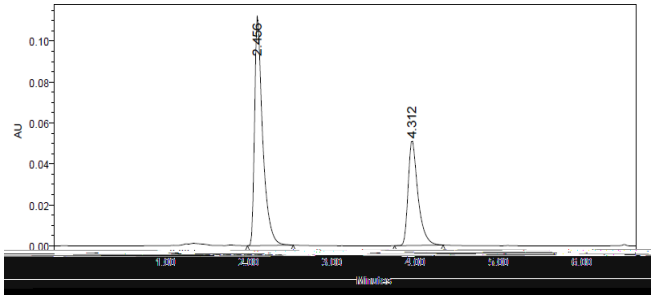

Figure 3: Typical chromatogram of Tezacaftor and Ivacaftor from standard solution

Method optimization

Different mobile phases were practiced to develop a liquid chromatographic method for the assay of Tezacaftor and Ivacaftor. The HPLC method was optimized through the evaluation of several solvent mixtures. A mobile phase of Methanol: Water 65:35 %v/v on Symmetry C18 4.6×150mm, 5µ column resulted in sharp, well-defined peaks with good resolution and low retention times were about 2.460 min for Tezacaftorand

4.312 min for Ivacaftor at the flow rate of 1 ml/min. The optimized chromatogram is shown in Figure 4 and the results are in Table 1. The % assay of drugs in the tablet dosage form was found to be 99.7% for Tezacaftor and 100.3% for Ivacaftor respectively. Results are shown in Table 2.

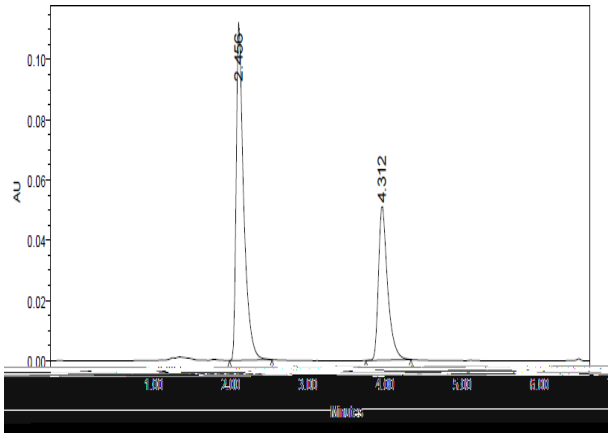

Figure 4: Chromatogram of Optimized condition

Table 1: System suitability results at optimized condition

S. No	Name of the component	Retention time(min)	Peak Area	Resolution	Tailing factor	Theoritical plates
1	Tezacaftor	2.460	600123	-	1.6	5011
2	Ivacaftor	4.315	422041	3.3	1.5	5947

Table 2: Assay Results of Tezacaftor and Ivacaftor

	Tezacaftor		Ivacaftor	
Injection No	**Standard area**	**Sample area**	**Standard area**	**Sample area**
1	600696	600269	422032	421047
2	600740	600272	422034	422042
3	600749	600275	422036	422041
4	600752	600279	422038	422042
5	600793	600275	422035	422040
Average	600746	600274	422034	421045
Tablet average weight 1.2g				
Standardweight 10mg				
Sampleweight 0.0369g				
Label amount	200mg		125mg	
Std.purity	99.7		99.7	
% Assay	99.7%		100.3%	

Table 3: System suitability results for Tezacaftor and Ivacaftor

Injection No	Tezacaftor				Ivacaftor			
	Rt (min)	**Peak Area**	**Plate count**	**Tailing factor**	**Rt (min)**	**Peak Area**	**Plate count**	**Tailing factor**
1	2.459	602561	5123	1.4	4.322	422674	5949	1.5
2	2.466	600543	5023.2	1.4	4.323	424692	5890.0	1.5
3	2.472	601288	5061.3	1.3	4.342	421255	5952.5	1.4
4	2.452	600776	5147.3	1.6	4.300	415235	5926.4	1.50
5	2.450	600758	5101.8	1.7	4.295	416260	5898.5	1.49
Mean	**2.459**	**601185.2**			**4.316**	**420023.2**		
SD	**0.006**	**816.3576**			**0.038**	**724.7845**		
%RSD	**0.7**	**0.13**			**0.8**	**0.17**		

Method validation

System suitability

After equilibration of the column with mobile phase, five replicates of 10µl standard solutions were injected. The System suitability of the method was evaluated using parameters from the recorded chromatograms. The % RSD of replicating injections was less than 1%. The system suitability results are publicized in Table 3.

Specificity

The specificity of the process was evaluated by injecting blank, sample, and standard preparations into the chromatographic system and chromatograms were compared. It was observed that there was no interference due to excipients from the tablet dosage form and from solvent at the retention time of analytes peaks and furthermore peaks showed good resolution. The chromatogram of blank preparation is in Figure 5

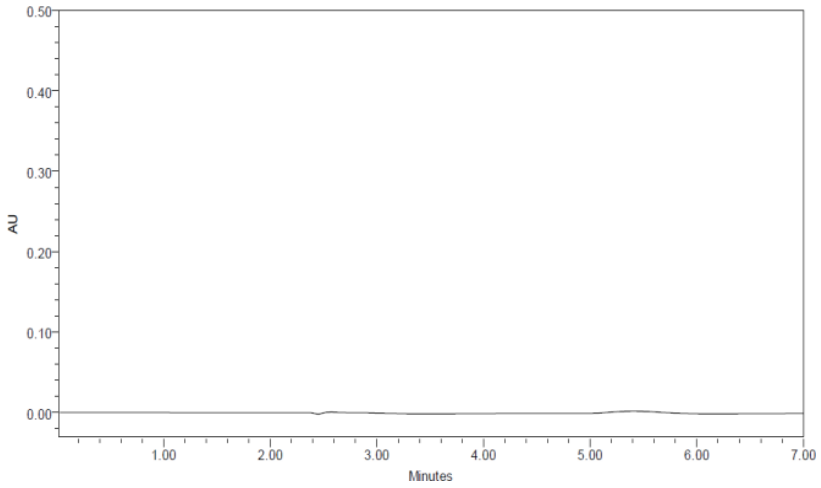

Figure 5: Chromatogram of blank preparation

Linearity

The linearity of response (peak area) for Tezacaftor and Ivacaftor was determined in a concentration range of 45- 225µg/ml for Tezacaftor and 10-50µg/ml for Ivacaftor. Each concentration level was injected in replicate into the HPLC system. The linearity was evaluated by the value of the correlation coefficient. The Linear regression coefficient for both drugs was 0.999 and good correlationwas obtained between the peak area and concentration as in Figures 6 and 7 and the results are shown in Table 4.

Table 4: Linearity results for Tezacaftor and Ivacaftor

	Tezacaftor		Ivacaftor	
S. No	**Concentration (µg/ml)**	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
1	45	215760	10	145474
2	95	417001	20	279372
3	135	600435	30	421045
4	180	791969	40	562151
5	225	974736	50	721671
Slope		**9693**		**14286**
Y-intercept		**15288**		**2194**
Correlation coefficient (R2)		**0.999**		**0.999**

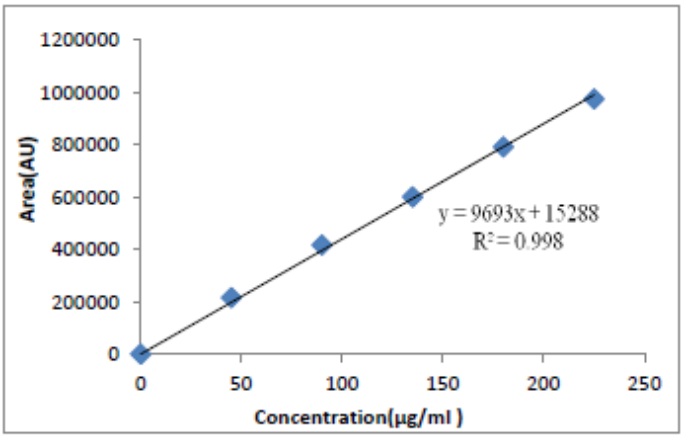

Figure 6: Calibration curve of Lumacator

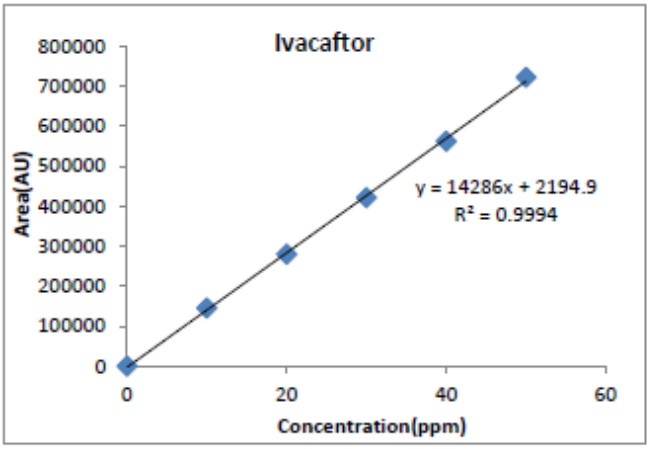

Figure 7: Calibration curve of Ivacaftor

Accuracy

The accuracy of the method was determined by recovery experiments and was performed by the standard addition method at three concentration levels of 50%, 100%, and150%. Each concentration was injected thrice into the chromatographic system and the percentage and mean % recoveries for both drugs were calculated and the results are given away in Table 5.

Precision

The precision of the method was evaluated by repeatability and intermediate precision studies. Repeatability was assessed by six replicates of 100% accuracy and Intermediate precision (inter-day precision) was evaluated by assaying six injections of sample solutionfollowingthedescriptionoftheanalytical method by different analysts on different days using different HPLC and columns of the similar make but dissimilar lot number. The % RSD for the response factor of both drugs was found to be less than 2% and results are revealed in Tables 6 and7

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and quantification was calculated from the signal to noise ratio. This ratio forLODis3:1andLOQis10:1.The limitofdetectionand quantification was evaluated from the calibration curves by applying statistical calculations and results are shown in Table8.

The limit of detection and quantification were expressed as:

$LOD=3.3\sigma/S$

$LOQ=10.0\ \sigma/S$ Where;

σ = Standard deviation of the response S = Slope of the regression line

Table 5: Recovery results of Tezacaftor and Ivacaftor

	Tezacaftor					**Ivacaftor**				
Accuracy Level (%)	**Amount added (in µg)**	**Amount recovered (in µg)**	**% Recovery**	**Mean % recovery**	**%RSD**	**Amount added (in µg)**	**Amount recovered (in µg)**	**% Recovery**	**Mean % recovery**	**%RSD**
50	30	30.3	100.6	100.1	0.4	15	14.9	99.3	99.9	0.5
50	30	30.1	100.3			15	15.01	100		
50	30	29.9	99.6			15	15.1	100.6		
100	60	60	100	100.3	0.3	30	29.9	99.6	100.0	0.3
100	60	60.5	100.8			30	30.07	100.2		
100	60	60.1	100.1			30	30.1	100.3		
150	90	90.2	100.2			45	44.8	99.5		
150	90	89.9	99.8	99.9	0.2	45	45.06	100.1	99.8	0.2
150	90	89.8	99.7			45	44.95	99.8		

Table 6: Repeatability Results of Tezacaftor and Ivacaftor

	Tezacaftor		Ivacaftor	
Injection No	**Rt (min)**	**Peak Area**	**Rt (min)**	**Peak Area**
1	2.453	603403	4.289	429183
2	2.455	608107	4.309	416643
3	2.453	607266	4.306	424052
4	2.452	608776	4.300	425235
5	2.450	609758	4.295	416260
6	2.451	607962	4.305	427183
Mean	**2.452**	**607545**	**4.3**	**423092**
SD	**0.004**	**2005.67**	**0.000**	**683.35**
%RSD	**0.16**	**0.33**	**0.001**	**0.16**

Robustness

Robustness of the method was demonstrated by analyzing the system suitability parameters under intentionally modified chromatographic conditions such as flow rate, and mobile phase ratio on the lower and higher side of the normal values. There was no considerable change in the retention time between the original method and modifications to the method. The results are illustrated in Table9.

Table 7: Intermediate precision Results of Tezacaftor and Ivacaftor

	Tezacaftor				Ivacaftor			
Injection No	**Rt (min)**	**Peak Area**	**Plate count**	**Tailing factor**	**Rt (min)**	**Peak Area**	**Plate count**	**Tailing factor**
1	2.465	602386	5075.9	1.5	4.323	422252	5886.2	1.6
2	2.472	608118	5043.2	1.3	4.343	418090	5947.5	1.5
3	2.467	605566	5029.9	1.5	4.324	424361	5907.8	1.55
4	2.466	608543	5023.2	1.4	4.323	424692	5890.0	1.50
5	2.472	609288	5061.3	1.4	4.322	411255	5852.5	1.49
6	2.476	607315	5078.4	1.3	4.323	422252	5756.8	1.50
Mean	**2.469**	**606869.3**			**4.326**	**420483**		
SD	**0.004**	**2538.025**			**0.0074**	**5096.97**		
%RSD	**0.16**	**0.41**			**0.17**	**1.2**		

Table 8: LOD and LOQ results for Tezacaftor and Ivacaftor

Name of the analyte	LOD µg/ml	LOQ µg/ml
Tezacaftor	0.83	2.5
Ivacaftor	1.3	3.95

Table 9: System suitability results for robustness study of Tezacaftor and Ivacaftor

Robust condition	Tezacaftor				Ivacaftor			
	Rt (min)	Peak Area	Tailing	Plate count	Rt (min)	Peak Area	Tailing	Plate count
Normal	2.456	600122	1.8	5215	4.312	422042	1.5	5648
Flow rate 0.9 ml	2.741	651206	1.79	5199	4.830	453012	1.6	5687
Flow rate 1.1 ml	2.270	546820	1.8	5234	3.979	398654	1.5	5602
Mobile phase 60:40v/v	3.266	586420	1.8	5298	3.828	445983	1.55	5643
Mobile phase 70:30v/v	2.147	542813	1.76	5287	2.257	402315	1.51	5699

CONCLUSION

A new simple, precise, and accurate analytical method has been urbanized for the simultaneous estimation of Ivacaftor and Tezacaftor in tablet dosage form by RP- HPLC. The optimum wavelength for the determination of ICF and LMF was selected at 270 nm based on Isobestic point. Various trials were performed in different ratios of methanol and water, but in the proportion of 65:35 %v/v methanol and water have been chosen as an ideal solvent system as a good peak symmetry and resolution between the peaks were observed. The Retention time of LMF and ICF was found to be 2.460 min and 4.312 min respectively. The retention time for both the drugs was appreciably less compared to the retention time obtained in the other ratios of the mobile phase. The different analytical presentation parameters such as linearity, accuracy, precision, LOD, LOQ, and specificity were determined as per the International Conference on Harmonization ICH Q2 (R1) guidelines. The method was specific as no interference was observed at a retention time of analytes from the solvent and from tablet excipients. Linear calibration curves were observed over the concentration range of 45-225 µg/ml for LMF and10-50 µg/ml for ICF. From linearity, the correlation coefficient (R2) value for both drugs was found to be 0.999. The number of theoretical plates was found to be more than 2000, which indicates the efficient performance of the column. The percentage of recovery of LMF and ICF was found to be 100.3% and 100% shows that the proposed method is highly accurate. The % RSD for precision was found to be <2. From this experimental and validation results, the developed method for the

simultaneous estimation of Tezacaftor and Ivacaftor in their combined dosage form by RP-HPLC was found to be simple, highly sensitive, precise, accurate, and high resolution. Also, the lower solvent consumption and shorter retention time lead to more acceptable, cost effective and Eco- friendly chromatographic procedures. Hence, it can be conveniently adopted for routine analysis of API content in the commercial formulations of Tezacaftor and Ivacaftor in Educational institutions and Quality control laboratories.

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