IJMPR 2022, 6(7), 65-71

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

DEVELOPMENT AND VALIDATION OF NOVEL RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IVACAFTOR AND TEZACAFTOR IN PHARMACEUTICAL FORMULATION

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Received on: 09/06/2022
Revised on: 29/06/2022
Accepted on: 19/07/2022

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ABSTRACT

Purpose: The objective of the proposed method is to develop a validated RP-HPLC method for simultaneous estimation of Ivacaftor and Tezacaftor from a fixed dose combination drug product. **Method:** The chromatographic condition for detection was developed using column of phenomenex C_{18} with dimensions of 250 x 4.6 mm, 5µm with mobile phase containing buffer 0.1M potassium dihydrogen phospahte and acetonitrile taken in the ratio of 60:40 was pumped through column at a flow rate of 1 ml/min. The temperature was maintained at 30°C with wavelength of 270 nm and detected by using PDA detector. **Results:** The retention time of Ivacaftor and Tezacaftor was found to be 2.218 min and 2.813 min respectively. %RSD of the Ivacaftor and Tezacaftor was and found to be 0.8 and 0.9 respectively. Accuracy studies were done with % recovery obtained as 100.35% and 100.69% for Ivacaftor and Tezacaftor respectively. LOD, LOQ values for Ivacaftor and Tezacaftor were 0.38 µg/mL, 1.14µg/mL and 0.09 µg/mL, 0.28 µg/mL respectively. Retention times was less when compared to the reported methods. **Conclusion:** The developed method was simple, precise, accurate, linear, rapid, economical and the method has the ability to separate both drugs in pharmaceutical dosage forms so it can be adopted in regular quality control test for the simultaneous estimation of Ivacaftor and Tezacaftor in tablet dosage form.

KEYWORDS: Ivacaftor (IVA), Tezacaftor (TEZ), RP-HPLC, simultaneous estimation.

1. INTRODUCTION

Ivacaftor **(Fig 1a)** is chemically known as N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide), is a transmembrane conductance regulator (CFTR) potentiator. It is used to improve breathing, reduce the risk of lung infections, and improve weight gain. Tezacaftor **(Fig 1b)** 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. These drugs are being used either alone or in combination for the treatment of cystic fibrosis in certain people with an abnormal "Cystic fibrosis transmembrane conductance regulator CFTR" gene.[1-2]

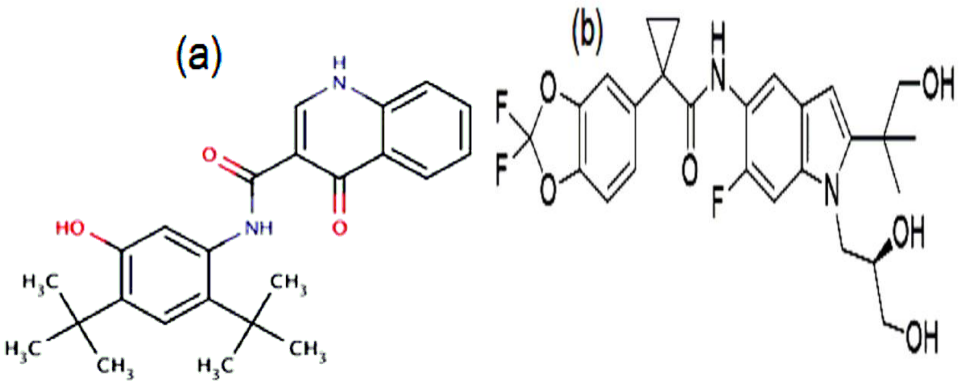

Fig. 1: Structure of a) Ivacaftor and b) Tezacaftor.

Literature survey revealed that Ivacaftor was estimated individually by UV[3], HPLC[4] method, whereas no method was reported for Tezacaftor. But there are methods reported for simultaneous estimation of Ivacaftor and Tezacaftor by UV[5], HPLC[6], UPLC[7,8] method in a combination dosage form. The aim of the current work is to develop a novel RP- HPLC method and validate according to ICH guidelines[9,10], for the method to be specific, precise, accurate, robust and sensitive for simultaneous determination of Ivacaftor and Tezacaftor in pure drug and marketed formulations.

2. MATERIALS AND METHODS

Chemicals and Reagents

Ivacaftor and Tezacaftor pure drugs were obtained from Mylan Labs, Hyderabad. Combination Ivacaftor and Tezacaftor tablets were procured from local pharmacy. HPLC grade water, Acetonitrile (HPLC grade), Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho Phosphoric acid were procured from Rankem chemicals.

Instrumentation: Analysis was performed on Waters HPLC 2695 series system equipped with quaternary pumps, PDA detector, an auto sampler and Phenomenex C18 (4.6 x 250mm, 5µm) compartment with Empower 2 software, Ultrasonic bath (BVK enterprises, India) Digital weighing balance (Denver company TR-203), digital pH meter (pH 500) and nylon syringe filter (Millipore, India) were used.

Chromatographic conditions

The chromatographic separation with good retention time was achieved on Phenomenex C18 (4.6 x 250mm, 5µm) column. Mobile phase composed of 0.1M potassium dihydrogen phosphate and acetonitrile in the ratio of 60:40 v/v with a flow rate of 1ml/min and run time of 5min. The detection of two drugs were observed at a wavelength of 270nm using PDA detector. The injection volume was 10µl and the temperature was maintained at 30°C.

Preparation of Standard stock solution

Accurately weighed 60 mg of Ivacaftor and 40 mg of Tezacaftor are transferred to individual 100 ml volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (600µg/ml of Ivacaftor and 400µg/ml of Tezacaftor).

Preparation of Standard working solution

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent gave 60µg/ml of Ivacaftor and 40µg/ml of Tezacaftor.

Preparation of Sample stock solution

20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 25ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters gave 600µg/ml of Ivacaftor and 400µg/ml of Tezacaftor.

Preparation of Sample working solution

1ml of filtered sample stock solution was transferred to 10mL volumetric flask and made up with diluent. (60µg/ml of Ivacaftor and 40µg/ml of Tezacaftor).

Method Validation

The method was validated for parameters like system suitability, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), specificity and robustness.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of 60µg/mL of Ivacaftor and 40µg/mL of Tezacaftor and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity

By appropriate aliquots of the standard Ivacaftor and Tezacaftor, six working solutions ranging between 15-90 µg/mL and 10-60 µg/mL were prepared. Linearity was performed in triplicate by injecting 10µL of solution.

Accuracy

The accuracy is the closeness of the test results obtained by that method to the true value. It is done by measuring the amount of pure drug recovered at three different concentrations at 50%, 100% and 150% in triplicate.

Precision

From a single volumetric flask of working standard solution of 60µg/mL of Ivacaftor and 40µg/mL of Tezacaftor, six injections were given within the day at time intervals and on different days and the corresponding areas were measured.

LOD and LOQ

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation(σ) and slope(S) was adopted.

$LOD = 3.3\ \sigma/S$

$LOQ = 10\ \sigma/S$

Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature were made. Robustness conditions like Flow rate of ±0.1 mL/min, mobile phase ratio of ±5%v/v, temperature of ±5°C was maintained and samples were injected in triplicate manner.

Specificity

Specificity of the method was determined by injecting blank and placebo to check whether peaks in the blank and placebo were eluting with drugs peaks.

3. RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

Spectroscopic analysis of compounds showed the isobestic point was at 270 nm for Ivacaftor and Tezacaftor. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. The optimized mobile phase was determined as a mixture of Potassium di hydrogen phosphate: Acetonitrile (60:40) at flow rate of 1mL/min and Ivacaftor and Tezacaftor were eluted at 2.223 and 2.885 min respectively.

Method Validation: System suitability parameters

Sample solution and six replicate injections were injected from freshly prepared standard solutions of IVA and TEZ. Each solution was analysed for their peak area, theoretical plates, resolution, and tailing factor. The optimized chromatogram was shown in Fig. 2 and all the system suitability parameters were represented in Table 1.

Table 1: System Suitability Parameters.

S. No	Ivacaftor			Tezacaftor			
Injection	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.218	7732	1.28	2.879	9400	1.2	6.0
2	2.222	7779	1.28	2.883	9980	1.19	5.8
3	2.224	7158	1.24	2.884	10319	1.19	5.8
4	2.224	7015	1.28	2.886	11105	1.18	5.9
5	2.234	7728	1.21	2.924	9808	1.21	6.3
6	2.289	7216	1.3	2.998	9587	1.21	6.0

Linearity

Six linear concentrations of IVA (15-90µg/ml), and TEZ (10-60µg/ml) were injected in a triplicate. Regression equations obtained for IVA was y = 26028xx + 4696.6, and TEZ was y = 23627x + 2971. Correlation coefficients of two drugs were found to be 0.999.The linearity plots were shown in Fig. 2 & 3.

Table 2: Linearity data for Ivacaftor and Tezacaftor.

Ivacaftor		Tezacaftor	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
15	402410	10	241770
30	786734	20	474925
45	1165461	30	706333
60	1575220	40	956078
75	1957570	50	1192093
90	2344330	60	1411241

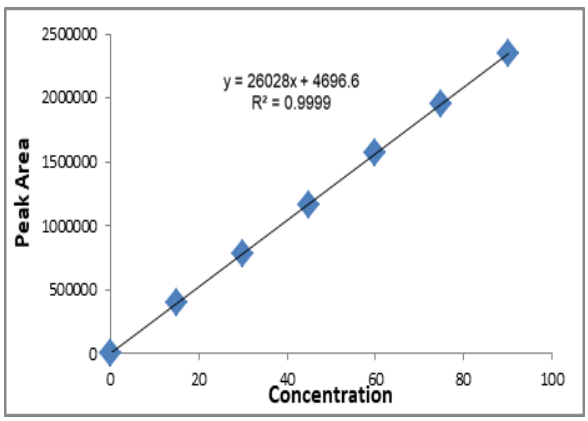

Fig. 2: Linearity plot for IVA.

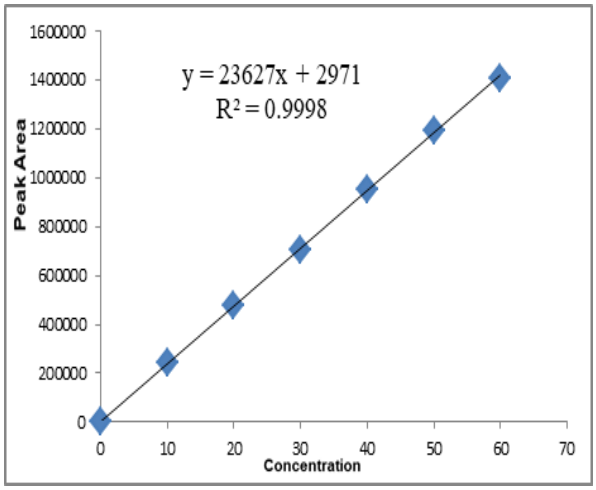

Fig. 3: Linearity plot for TEZ.

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 100.35% and 100.69% for Ivacaftor and Tezacaftor respectively. The results were given in Table 3 and 4. It shows that the developed method was accurate.

Table 3: Accuracy results for IVA and TEZ.

Drug Name	% Level	Amount taken	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
IVA	50%	60	30	91.79	101.99	100.5%
		60	30	88.99	98.88	
		60	30	90.57	100.64	
	100%	60	60	121.15	100.96	100.3%
		60	60	119.512	99.59	
		60	60	120.564	100.47	
	150%	60	90	149.226	99.48	100.31%
		60	90	151.751	101.47	
		60	90	149.98	99.99	
TEZ	50%	40	20	58.89	98.16	100.143%
		40	20	60.62	101.64	
		40	20	60.47	100.78	
	100%	40	40	81.54	101.94	101.596%
		40	40	80.99	101.24	
		40	40	81.28	101.61	
	150%	40	60	101.57	101.57	100.49%
		40	60	99.75	99.78	
		40	60	100.14	100.14	

Precision

For inter-day precision %RSD values obtained were 0.8% and 0.9% respectively for Ivacaftor and Tezacaftor. % RSD for intraday precision obtained were 0.8% and 0.9% respectively for Ivacaftor and Tezacaftor. As the limit of Precision was less than "2" precision was passed for the method. The % RSD for intraday and interday precision were calculated for two drugs as shown in Table 5 and 6.

Table 5: Intra-Day precision data for Ivacaftor and Tezacaftor.

S. No	Area of Ivacaftor	Area of Tezacaftor
1.	1615103	977378
2.	1624387	985152
3.	1600006	970599
4.	1601721	971171
5.	1600097	963379
6.	1631506	983380
Mean	1611543	975177
S.D	13672.1	8340.7
%RSD	0.8	0.9

Table 6: Interday precision data for Ivacaftor and Tezacaftor.

S. No	Area of Ivacaftor	Area of Tezacaftor
1.	1615166	983465
2.	1560931	969094
3.	1585653	947490
4.	1555232	947142
5.	1606725	962106
6.	1562963	944093
Mean	1581112	958898
S.D	25466.7	15526.2
%RSD	1.6	1.6

LOD and LOQ

LOD and LOQ for Ivacaftor was 0.38 µg/mL and 1.14 µg/mL respectively and for Tezacaftor was 0.09 µg/mL and 0.28 µg/mL respectively. It shows that the method was sensitive.

Robustness

Small deliberate changes in method like flow rate, mobile phase ratio, and temperature were made. There was no recognized change in the peak area and were within range as per ICH Guide lines and shown in Table 7.

Table 7: Robustness data for Ivacaftor and Tezacaftor (n=3).

S.no	Parameters	%RSD of Ivacaftor (Peak Area)	%RSD of Tezacaftor (Peak Area)
1	Flow rate of 0.9ml/min	0.3	0.3
2	Flow rate of 1.1ml/min	0.8	0.6
3	Mobile phase ratio of 65B:35A	0.2	0.2
4	Mobile phase ratio of 55B:45A	0.4	0.4
5	Temperature of 25°C	0.4	0.4
6	Temperature of 35°C	1.2	1.3

*Note: B=Buffer, A=Acetonitrile

Specificity

The method was found to be specific as blank and placebo did not interfere with the drug peaks in the chromatogram shown in Fig.4 and 5.

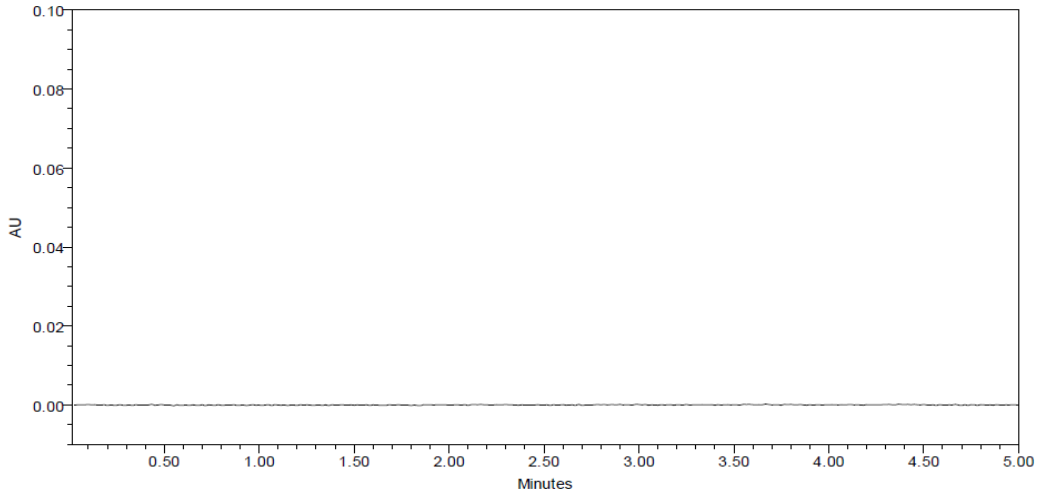

Fig.4: Chromatogram of blank.

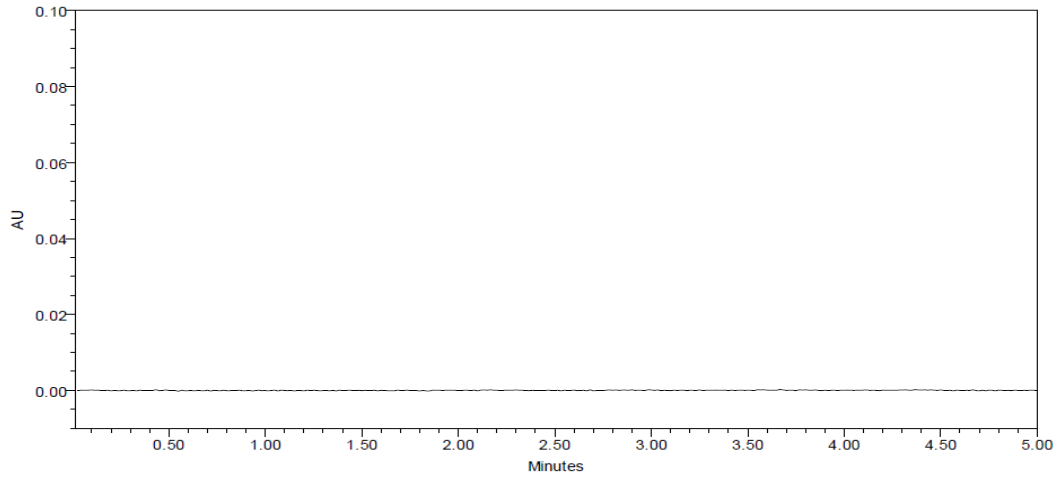

Fig. 5: Chromatogram of placebo

Assay

SYMDEKO, the marketed formulation with the label claim Ivacaftor 150mg, Tezacaftor 100mg was performed with the above formulation. Average % Assay for Ivacaftor and Tezacaftor obtained was 100.82% and 100.89% respectively were shown in Table 8. The standard and sample chromatogram was shown in Fig.6 and 7.

Table 8: Assay of Ivacaftor and Tezacaftor.

	Assay of Ivacaftor			Assay of Tezacaftor		
S.No	**Standard Area**	**Sample area**	**% Assay**	**Standard Area**	**Sample area**	**% Assay**
1	1615103	1629420	100.91	977378	992058	101.53
2	1624387	1626262	100.71	985152	984877	100.79
3	1600006	1636635	101.35	970599	986859	101.00
4	1601721	1635464	101.28	971171	990697	101.39
5	1600097	1630581	100.98	963379	983667	100.67
6	1631506	1609817	99.69	983380	976901	99.98
Avg			**100.82**			**100.89**

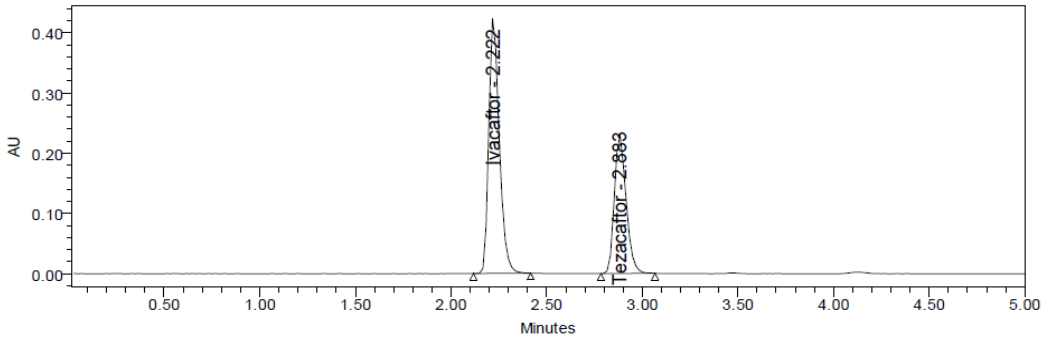

Fig. 6: Chromatogram of working standard solution of Ivacaftor and Tezacaftor.

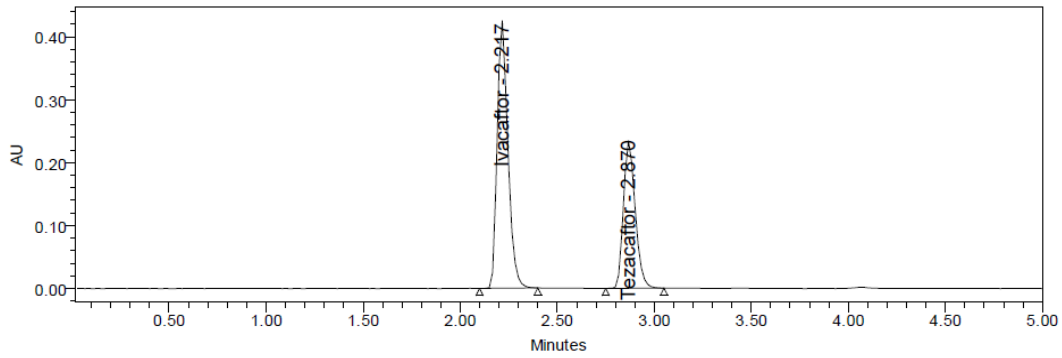

Fig. 7: Chromatogram of working sample solution of Ivacaftor and Tezacaftor

4. CONCLUSION

The developed RP-HPLC method was found to be accurate, precise, robust, sensitive and specific. The method was successful in simultaneous estimation of Ivacaftor and Tezacaftor in bulk drug and pharmaceutical formulations. So, the present RP-HPLC method is suitable for ascertaining the quality control of the raw materials, formulation in combined dosage form and dissolution studies.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ACKNOWLEDGEMENT

The authors thank to Dr. G. Tulja Rani, Principal of Malla Reddy Pharmacy College, Hyderabad, for providing all necessary facilities for this project work.

FUNDING SOURCE

There is no source of funding.

DATA AVAILABILITY

Not declared.

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