ORIGINAL ARTICLE

Development and validation of reversed-phase high-performance liquid chromatography method for the simultaneous determination of tezacaftor and ivacaftor in bulk and pharmaceutical formulation

G. Dharmamoorthy, K. Chandrasukeerthi, M. Kishore Babu, M. Gurava Reddy, K. V. Nanda Kumar

Department of Pharmaceutical Analysis, Krishna Teja Pharmacy College, Renigunta, Tirupathi, Andhra Pradesh, India

Abstract

Objective: The objective of the study was to develop analytical method for the estimation of tezacaftor and ivacaftor in bulk and its combination dosage form and to validate the method developed according to ICH guidelines. **Materials and Methods:** Chromatographic separation was achieved through Ascentis C18 150 × 4.6 mm, 5 m using 0.1% OPA: acetonitrile (70:30 v/v) mixture used as the mobile phase. The Water ACQUITY Model high-performance liquid chromatography system with photodiode array detector and EMPOWER version 2.0 software was monitored at detection wavelength 260 nm with flow rate of 1.0 ml/min and the method was validated as per ICH guidelines. **Results and Discussion:** By applying the proposed method, the retention times of tezacaftor and ivacaftor were found to be 3.0 and 3.6 min, respectively. %RSD of method precision for tezacaftor and ivacaftor was found to be 0.8 and 0.5, respectively. % recovery was obtained as 99.61% and 99.92% for tezacaftor and ivacaftor, respectively. Retention times were less so, the method developed was simple and economical and can be adopted in regular quality control analysis for selected drugs. **Conclusion:** This method was successfully applied for the determination of tezacaftor and ivacaftor in their pharmaceutical formulation and hence can be the routine analysis of these drugs in combined dosage form.

Keywords: ICH guidelines, Reverse-phase high-performance liquid chromatography, Tezacaftor and Ivacaftor, Validation

INTRODUCTION

Tezacaftor chemically described as [Figure 1] 1-(2,2-dichloro-2H-1,3-benzodioxol-5-yl)-N- {1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-(1-hydroxy2-methylpropan-2yl)-1H-indol-5-yl]} cyclopropene-1-carboxamide. Its empirical formula is $C_{26}H_{27}F_3N_{206}$ and its molecular weight is 520.505. Ivacaftor chemically described [Figure 2] as N-2, 4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide. Its empirical formula is $C_{24}H_{28}N_{203}$ and molecular weight is 392.49.

Tezacaftor, in combination with ivacaftor, is indicated for the treatment of cystic fibrosis (CF) in people aged 12 years or older who have two copies of the F508del mutation or at least one mutation in the CF transmembrane conductance regulator (CFTR) gene that is responsive to this treatment based on clinical evidence.[1-3] Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of CF in patients aged 2 years

Address for correspondence: G. Dharmamoorthy, Department of Pharmaceutical Analysis, Krishna Teja Pharmacy College, Renigunta Road, Tirupati, Andhra Pradesh, India.
E-mail: dharmamoorthy111@gmail.com

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and older. CF is an autosomal recessive disorder caused by one of several different mutations in the gene for the CFTR protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes.[4-6]

From literature survey, various methods (Spectroscopic method: UV method, chromatographic method: High-performance liquid chromatography [HPLC] and UPLC) were reported for the analysis of individual drugs and also in combination with other drugs but no method was reported for simultaneous estimation of tezacaftor and ivacaftor. Hence, the purpose of the present work was to develop and validate reversed-phase HPLC (RP-HPLC) method for simultaneous estimation of tezacaftor and ivacaftor in bulk and its dosage form.

MATERIALS AND METHODS

Instruments Used

HPLC used for the study was equipped with Waters, photodiode array detector and autosampler integrated EMPOWER software, electronic balance, pH meter, ultrasonicator, and LABINDIA UV double-beam spectrophotometer with UV win 5 software. The chromatographic separation was achieved on a column Ascentis C18 150 mm × 4.6 mm, 5 m at a temperature of 30°C. Denver electronic analytical balance used for weighing purpose and pH meter was used to adjust the pH.

Drugs and Chemicals

Tezacaftor and ivacaftor drugs were kindly supplied by SS Pharma Labs, Guntur, India. Acetonitrile, triethylamine, potassium dihydrogen orthophosphate, and orthophosphoric acid are obtained from Rankem Chemicals. All chemicals are of analytical (AR) grade except acetonitrile which is a HPLC grade chemical.

Preparation of Stock Solution

Accurately weighed 5 mg of tezacaftor standard drug and 7.5 mg of ivacaftor standard drug standard drug were transferred into two 50 ml volumetric flasks separately. Ten milliliters of diluent were added to each flask and sonicated for 20 min. Flasks were made up to the mark with diluent and labeled as standard stock solution 1 and 2 (100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor).

Preparation of Mobile Phase

Mobile phase was prepared by mixing 0.1% OPA and acetonitrile in the ratio of 70:30 v/v.

Preparation of Sample Stock Solutions

Five Symkevi tablets were weighed individually and average weight of each tablet was calculated. Weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25 mL of diluent added and sonicated for 50 min, further the volume was made up to the mark with diluent and filtered (500 µg/ml of tezacaftor and 750 µg/ml of ivacaftor).

Preparation of Sample Working Solutions (100% Solution)

From the filtered solution, 0.2 ml was pipette out into a 10 ml volumetric flask and made up to 10 ml with diluents (10 µg/ml of tezacaftor and 15 µg/ml of ivacaftor).

Preparation of Spiked Solution

Preparation of 50% spiked solution

0.5 ml of standard stock solution (100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor) were taken into a 10 ml volumetric flask and made up to the mark with diluent (5 µg/ml of tezacaftor and 75 µg/ml of ivacaftor).

Preparation of 100% spiked solution

1.0 ml of standard stock solution (100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor) were taken into a 10 ml volumetric flask and made up to the mark with diluent (10 µg/ml of tezacaftor and 15 µg/ml of ivacaftor).

Preparation of 150% spiked solution

1.5 ml of standard stock solution (100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor) were taken into a 10 ml volumetric flask and made up to the mark with diluent (15 µg/ml of tezacaftor and 22.5 µg/ml of ivacaftor).

Selection of Detection Wavelength

Both the drugs tezacaftor and ivacaftor were scanned in the wavelength region of 200–400 nm using photodiode array detector. It was found that both the drugs have shown good peak response at a detection wavelength of 260 nm. Therefore, 260 nm was selected as detection wavelength in the present study.

Method Development

Method development was done by changing various columns, mobile phase ratios, buffers and its pH, etc. tezacaftor and ivacaftor were eluted with good peak shape here the representive optimized chromatogram present in the Figure 3.

Optimized Chromatographic conditions

Mobile phase: 0.1%0PA: acetonitrile (70:30v/v)

Flow rate: 1.0 ml/min
Column: Ascentis C18 (150 mm × 4.6 mm, 5.0 µm)
Detector wavelength : 260 nm
Column temperature: 30°C
Injection volume: 10 µL
Diluent: Water and acetonitrile in the ratio 50:50

RESULTS AND DISCUSSION

Tezacaftor and ivacaftor were eluted at 3.0 min and 3.6 min, respectively. Plate count and tailing factor were satisfactory. Hence, this method was optimized and selected for validation.

Method Validation

System suitability

The system suitability parameters were determined by preparing standard solutions of tezacaftor and ivacaftor and the solutions were injected 6 times and the parameters such as peak tailing, resolution, and USP plate count were determined.

The % RSD for the peak area of six standard injections results was found to be in Tables 1 and 2 and Figure 4.

Specificity

There should be no interfering peaks for blank and placebo samples at retention times of the selected drugs using the developed method to determine the specificity of the developed method. Specificity determined by black chromatogram, placebo chromatogram, and optimized chromatogram in Figures 5-7.

Linearity and Range

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. A series of working standard solutions were prepared in 50 mL calibrated volumetric flasks by appropriate dilution of the stock solution with mobile phase to obtain a concentration range of 2.5–15 µg/ml for tezacaftor and 3.75–22.5 µg/ml for ivacaftor. Each solution was injected 3 times and the chromatograms were recorded. Calibration curves for both the drugs were plotted by taking average peak area on the Y-axis and the concentration on the X-axis. The linearity data and the calibration curves are presented in Tables 3 and 4 and Figures 8 and 9.

Precision

Precision studies were performed by injecting 6 times standard solutions of tezacaftor and ivacaftor. Three types of precision, namely, system precision, repeatability, and intermediate precision were performed. The % RSD for the peak area of each six standard injections results was found in Tables 5 and 6 and Figure 10.

Table 1: System suitability of tezacaftor

S. No.	Tezacaftor		
Inj.	Rt (min)	TP	Tailing
1	3.0	4058	1.34
2	3.0	4268	1.35
3	3.0	4320	1.35
4	3.0	4211	1.35
5	3.0	3967	1.33
6	3.0	4191	1.34

Table 2: System suitability of ivacaftor

S. No.	Ivacaftor		
	Rt (min)	TP	Tailing
1.	3.6	5395	1.43
2.	3.6	5410	1.39
3.	3.6	5207	1.43
4.	3.6	5427	1.43
5.	3.6	5409	1.40
6.	3.6	5478	1.42

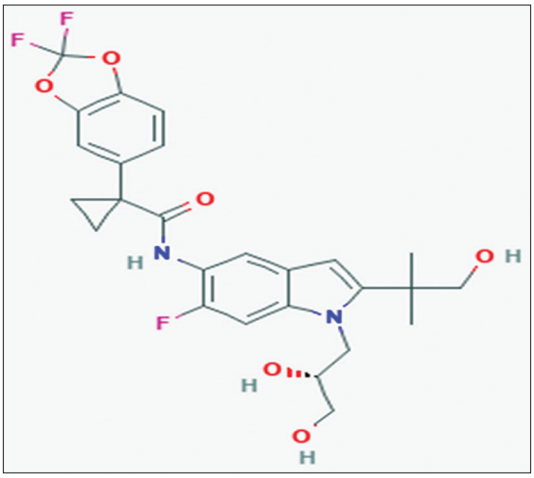

Figure 1: Chemical structure of tezacaftor

Accuracy

The accuracy of the method was determined by calculating recoveries of tezacaftor and ivacaftor by the method of standard addition. Known amount of tezacaftor and ivacaftor was added by triplicate injections at 50%, 100%, and 150% levels. All the prepared solutions were filled in separate HPLC vials. Each of these solutions was injected into the HPLC system and the chromatograms were recorded using optimized chromatographic conditions. The mean% recovery values and the %RSD values were calculated and given in Tables 7 and 8 and Figures 11-13.

Robustness

Robustness conditions such as flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus (65:35), mobile phase plus (55:45), temperature minus (25°C), and temperature plus (35°C) were maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Changes in results are shown in Table 9.

Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 3.3 \times SD/S$$

Where

SD – Standard deviation (SD) (obtained from method precision)

S – Slope

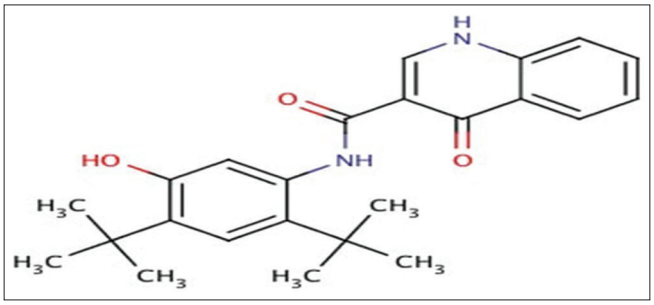

Figure 2: Chemical structure of ivacaftor

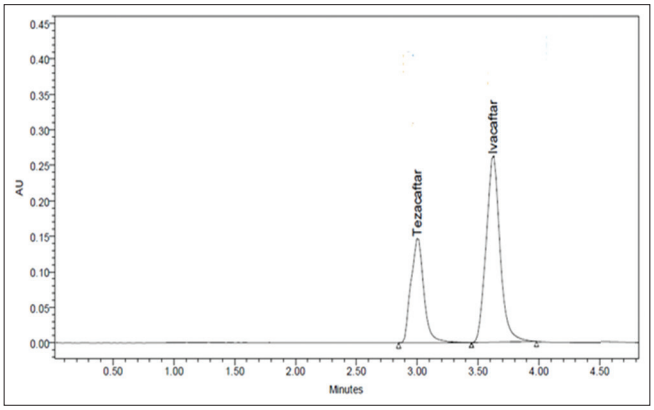

Figure 3: Optimized chromatogram

Table 3: Linearity table for tezacaftor

Tezacaftor	
Conc. (µg/mL)	**Peak area**
2.5	207,562
5	403,155
7.5	602,356
10	807,326
12.5	1,010,415
15	1,203,289

Table 4: Linearity table for ivacaftor

Ivacaftor	
Conc. (µg/mL)	**Peak area**
3.75	564,437
7.5	1,123,830
11.25	1,684,813
15	2,263,196
18.75	2,802,718
22.5	3,327,885

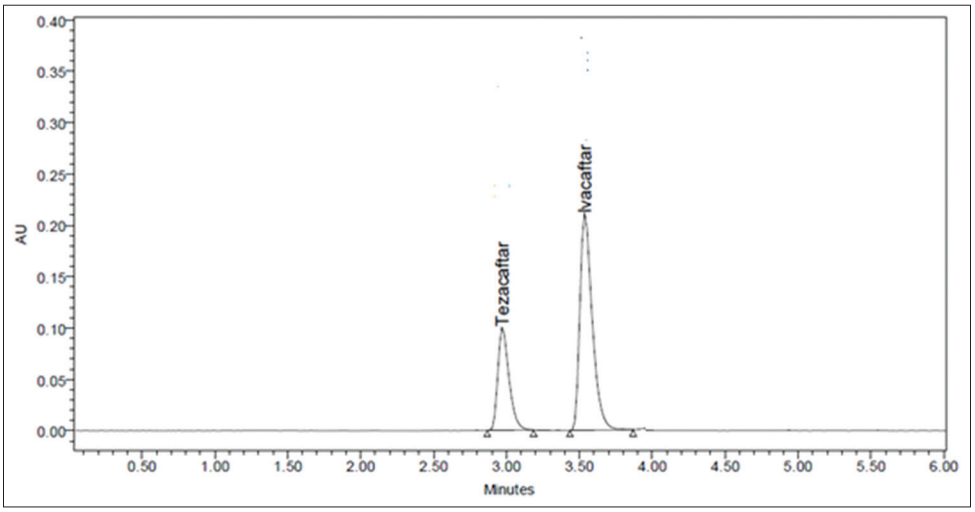

Figure 4: System suitability chromatogram

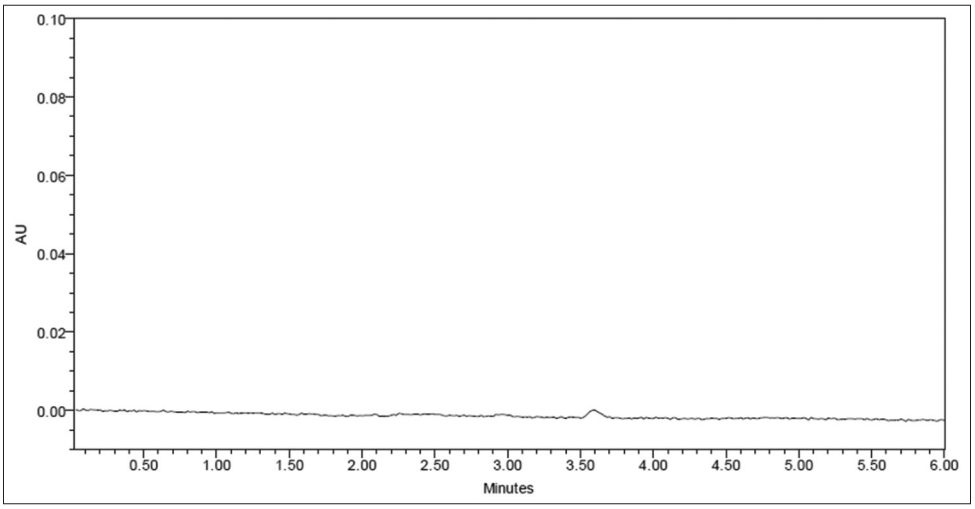

Figure 5: Blank chromatogram

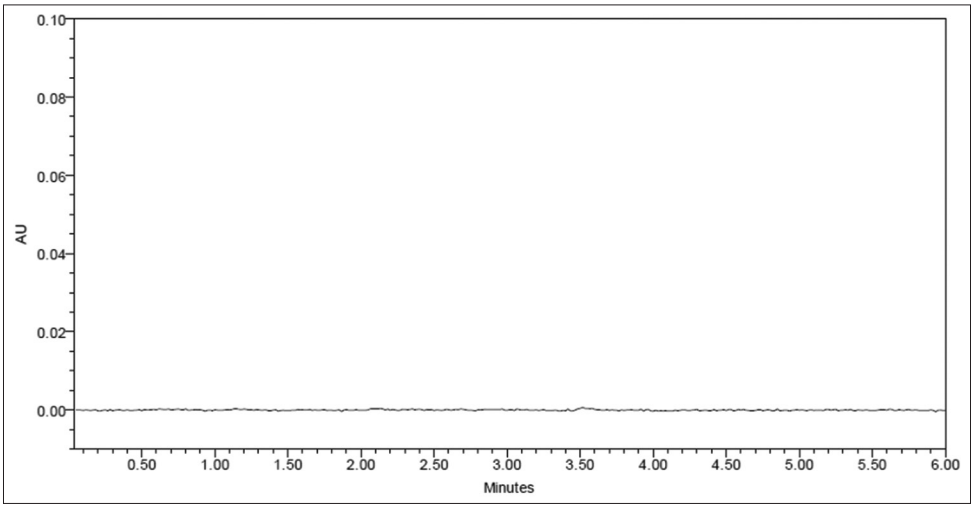

Figure 6: Placebo chromatogram

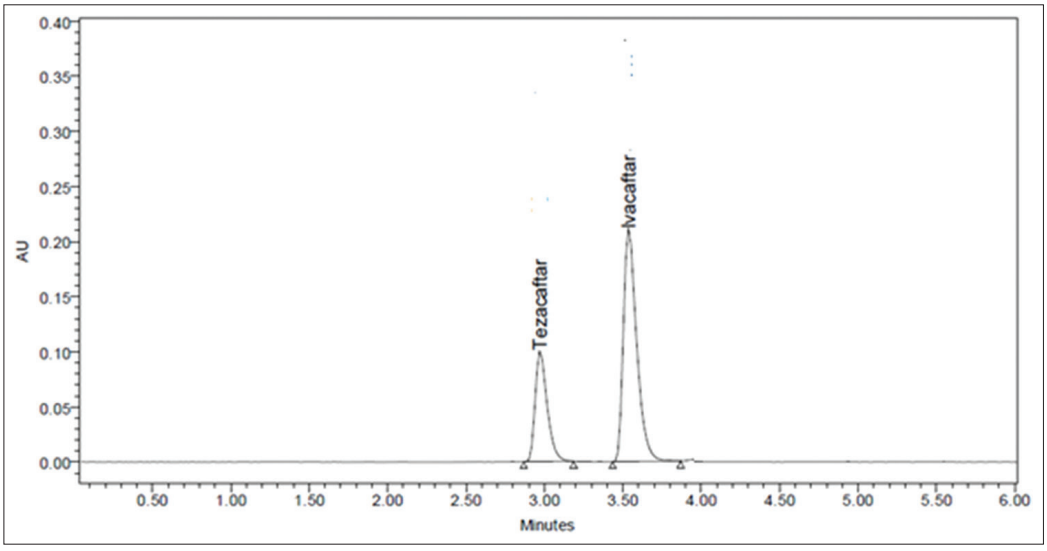

Figure 7: Optimized chromatogram

Limit of quantification (LOQ)

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 10 \times SD/S$$

Where

SD – Standard deviation (SD) (obtained from repeatability precision) and S – Slope.

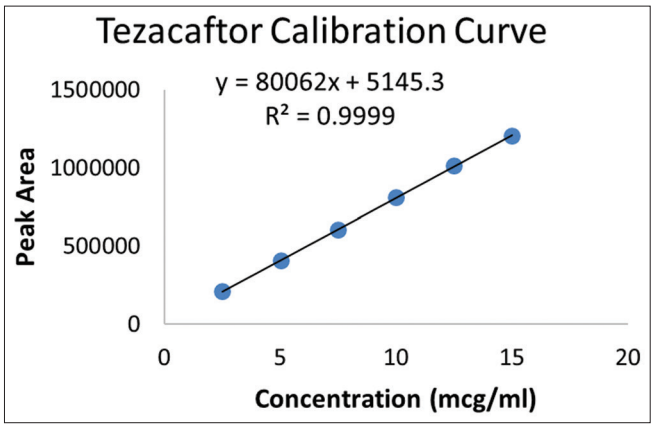

Figure 8: Calibration curve of tezacaftor

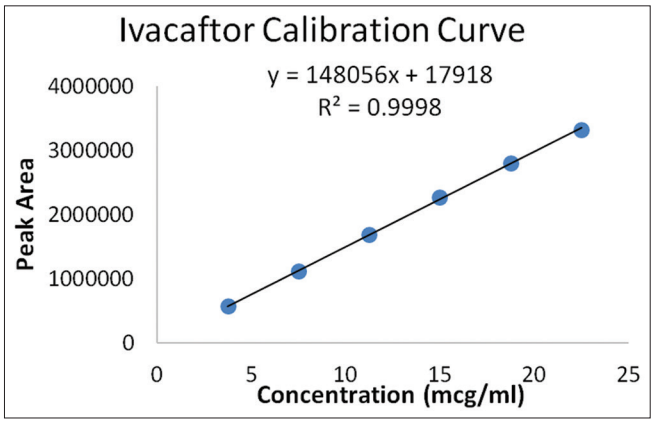

Figure 9: Calibration curve of ivacaftor

Table 5: Method precision table of tezacaftor

S. No.	Peak area of tezacaftor
1.	806,357
2.	808,057
3.	804,174
4.	790,645
5.	804,851
6.	808,891
Mean	803,829
S.D	6706.3
%RSD	0.8

Table 6: Method precision table of ivacaftor

S. No.	Peak area of ivacaftor
1.	2,246,978
2.	2,237,341
3.	2,248,021
4.	2,264,471
5.	2,243,697
6.	2,230,231
Mean	2,245,123
S.D	10,571
%RSD	0.4

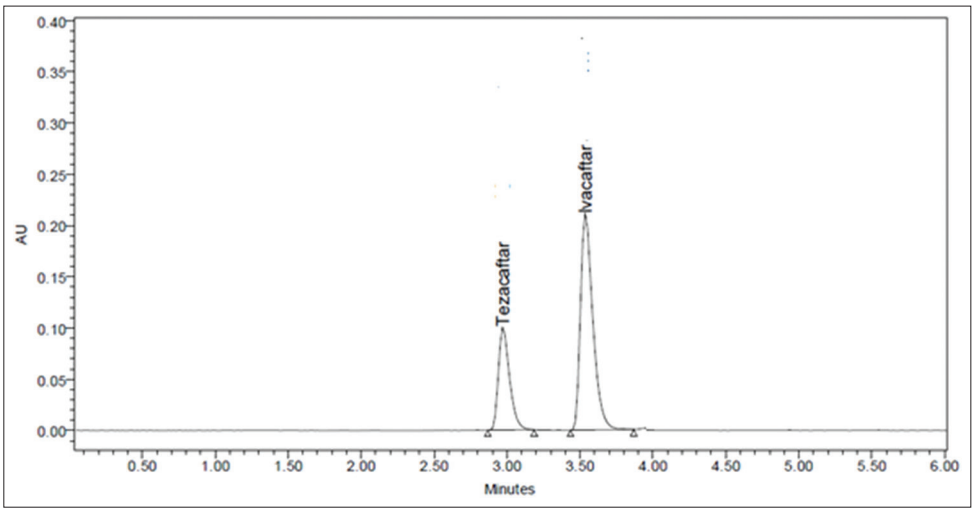

Figure 10: Method precision chromatogram

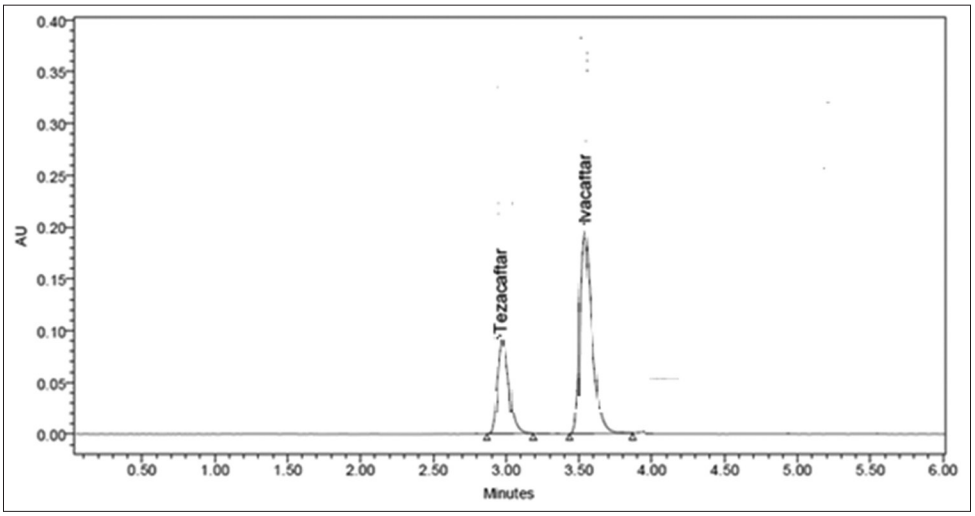

Figure 11: Accuracy 50% chromatogram

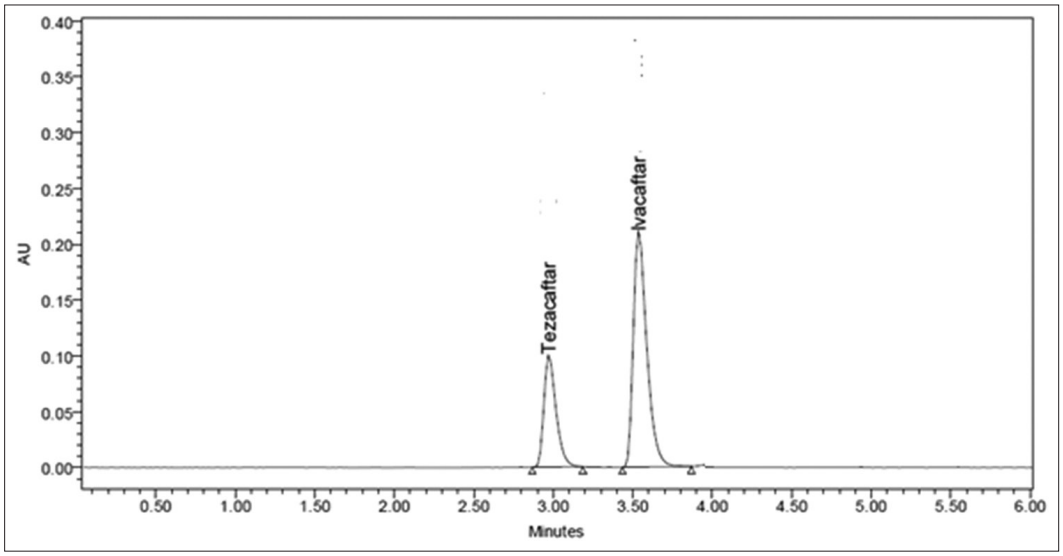

Figure 12: Accuracy 100% chromatogram

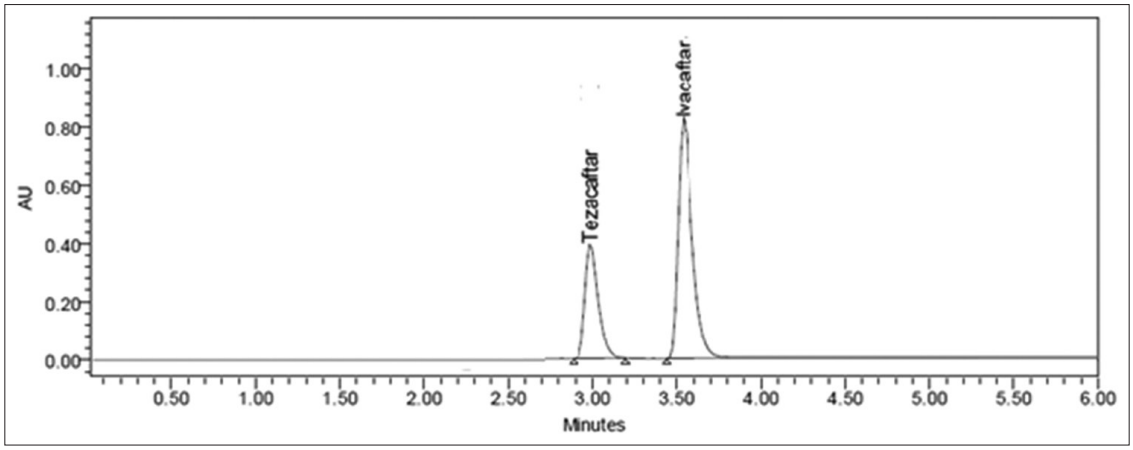

Figure 13: Accuracy 150% chromatogram

Table 7: Accuracy table of tezacaftor

% level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% recovery	Mean %recovery
50%	5	4.983	99.668	99.61%
	5	4.930	98.606	
	5	4.933	98.654	
100%	10	9.852	98.520	
	10	9.965	99.648	
	10	9.947	99.468	
150%	15	15.089	100.591	
	15	15.164	101.094	
	15	15.030	100.201	

Table 8: Accuracy table of ivacaftor

% level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% recovery	Mean %recovery
50%	7.5	7.39	98.50	99.92%
	7.5	7.48	99.72	
	7.5	7.36	98.18	
100%	15	15.14	100.91	
	15	15.07	100.48	
	15	15.16	101.04	
150%	22.5	22.40	99.58	
	22.5	22.57	100.30	
	22.5	22.63	100.57	

Sensitivity

The sensitivity values of tezacaftor and ivacaftor were found to be in Table 10.

Assay

Amount of drug present in the formulation was estimated by taking the standard as the reference. The average % was calculated and found to be 99.45% and 99.06% for tablet form of Tezacaftor and Ivacaftor respectively.Hemce the method was successfully employed for assay of tablet formulation.

Robustness

Table 9: Robustness data for tezacaftor and ivacaftor

S. No.	Condition	%RSD of tezacaftor	%RSD of ivacaftor
1	Flow rate (−) 0.9 ml/min	1.08	1.16
2	Flow rate (+) 1.1 ml/min	0.60	0.88
3	Mobile phase (−) 65:35	1.21	1.18
4	Mobile phase (+) 55:45	1.33	1.48
5	Temperature (−) 25°C	0.77	1.07
6	Temperature (+) 35°C	0.66	0.44

Sensitivity

Table 10: Sensitivity table of tezacaftor and ivacaftor

Drug	LOD (µg/ml)	Limit of quantification (µg/ml)
Tezacaftor	0.28 µg/ml	0.84 µg/ml
Ivacaftor	2.36 µg/ml	7.14 µg/ml

Assay data

Table 11: Assay data of tezacaftor

S. No.	Standard area	Sample area	% assay
1	806,357	797,575	99.09
2	808,057	794,718	98.73
3	804,174	808,055	100.39
4	790,645	801,225	99.54
5	804,851	807,323	100.30
6	808,891	794,341	98.68
Avg.	803,324	800,540	99.45
St. dev.	6706.3	6067.4	0.754
%RSD	0.8	0.8	0.8

Table 12: Assay data of ivacaftor

S. No.	Standard area	Sample area	% assay
1	2,286,978	2,212,660	98.50
2	2,237,341	2,219,443	98.81
3	2,228,021	2,223,570	98.99
4	2,234,471	2,244,491	99.92
5	2,203,697	2,226,605	99.12
6	2,260,231	2,224,123	99.01
Avg.	2,241,790	2,225,149	99.06
St. dev.	28,621.8	10,661.3	0.47
%RSD	1.3	0.5	0.5

DISCUSSION

By applying the proposed method, all the system suitability parameters were within the acceptance limits. Retention times of tezacaftor and ivacaftor were 3.0 min and 3.6 min, respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs using optimized method. Hence, this method was said to be specific. Average area, standard deviation, and % RSD were calculated for two drugs and obtained as 0.8% and 0.4%, respectively, for tezacaftor and ivacaftor. As the limit of precision was less than "2," the system precision was passed. Triplicate injections were given for each level of accuracy and mean % recovery was obtained as 99.61% and 99.92% for tezacaftor and ivacaftor. Robustness conditions such as flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus (65:35), mobile phase plus (55:45), temperature minus (25°C), and temperature plus (35°C) were maintained and samples were injected in duplicate manner. System parameters were passed. %RSD was within the limit. The label claim ivacaftor (150 mg) and tezacaftor (100 mg) per unit formulation assay was performed. Average %Recovery was obtained as 99.45% and 99.06% for ivacaftor and tezacaftor respectively.

CONCLUSION

Tezacaftor and ivacaftor were simultaneously determined in tablet matrix using RP-HPLC method. The method developed is simple, accurate, rapid, sensitive, and specific. RP-HPLC may be recommended for routine and quality control analysis of investigated drugs in two component pharmaceutical preparations. HPLC method found more precise, economical. The method was validated according to ICH guidelines.

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