IJPSR (2019), Volume 10, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 February 2019; received in revised form, 25 May 2019; accepted, 14 June 2019; published 01 November 2019

A NOVEL STABILITY INDICATING UPLC METHOD FOR THE ESTIMATION OF TEZACAFTOR AND IVACAFTOR IN TABLET DOSAGE FORM

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Keywords:

UPLC, Stability indicating assay method, Tezacaftor and Ivacaftor

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ABSTRACT: UPLC is a modern technique which refers to Ultra Performance Liquid Chromatography and enhances in three main areas: Speed, resolution, and sensitivity. In this present study, an accurate and precise UPLC method was developed and validated for the stability indicating assay method to estimate Tezacaftor and Ivacaftor simultaneously in both bulk and tablet dosage form. This method was developed using column HSS C18 (100 × 2.1mm 1.7µ) with mobile phase 0.1% OPA Buffer: acetonitrile taken in the ratio 50:50. The flow rate was 0.3 ml/min with an 1 µl injection volume. The effluents were detected at a wavelength of 292 nm using the TUV detector. The method was validated concerning specificity, accuracy, linearity, precision, robustness. The correlation coefficient for Tezacaftor and Ivacaftor were found to be 0.999 and 0.999, respectively. Recovery of Tezacaftor and Ivacaftor in the formulation was found to be 99.97% and 99.65% respectively.

INTRODUCTION: Ultra Performance Liquid Chromatography [1] (UPLC) applicable for particle less than 2 µm in diameter to acquire better resolution, speed, and sensitivity compared with high-performance liquid chromatography (HPLC). In twenty-first centenary pharmaceutical industries are focusing for new ways to in economy and shorten the time for the development of drugs. UPLC analysis in the meantime gives the better quality of their products, and analytical laboratories are not an exception in this trend. The separation and quantification in UPLC are done under very high pressure (up to 100M Pa).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(11).4968-73

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).4968-73

As compare to HPLC, under high pressure, it is observed that not any negative influence on the analytical column and also other components like time and solvent consumption is less in UPLC.

Combination of Tezacaftor and Ivacaftor indicated for the treatment of patients with cystic fibrosis. Cystic fibrosis [2] is a progressive, genetic disease that causes persistent lung infections and limits the ability to breathe over time. SYMDEKO is co-packaged as tezacaftor 100 mg/ivacaftor 150 mg fixed-dose combination tablets and ivacaftor 150 mg tablets. Chemical name of Tezacaftor is 1-(2, 2-difluoro-2H-1, 3- benzodioxol-5-yl)-N -{1-[(2R)-2, 3- dihydroxypropyl]-6- fluoro-2- (1-hydroxy-2-methylpropane-2-yl)- 1Hindol-5-yl} cyclopropane-1-carboxamide. Its molecular formula is $C_{26}H_{27}N_2F_3O_6$. Tezacaftor facilitates the cellular processing and trafficking of normal and select mutant forms of CFTR (including F508del-CFTR) to increase the amount of mature CFTR protein

delivered to the cell surface. Structure of Tezacaftor is shown in **Fig. 1**. Ivacaftor is a CFTR potentiator. Its chemical name is N-(2, 4-di-tert-butyl-5 -hydroxy phenyl)-1, 4-dihydro -4-oxyquinoline-3-carboxamide.Its molecular formula is $C_{24}H_{28}N_2O_3$. Structure of Ivacaftor is shown in **Fig. 2**.

Literature review reveals that only two works have been reported on a specified combination, i.*e.* UV [3] and HPLC [4, 5]. There are few methods for Ivacaftor in single [6] and with other drug combination for HPLC [7, 8] and LC/MS [9]. However, stability indicating UPLC method was not available.

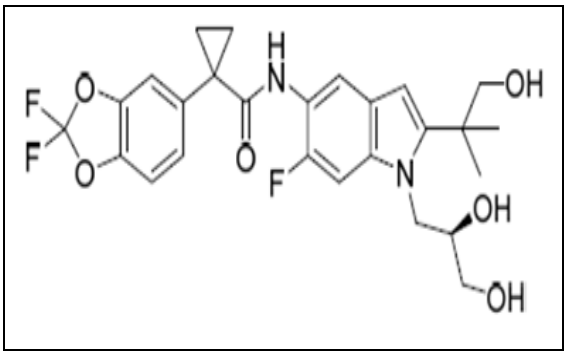

FIG. 1: STRUCTURE OF TEZACAFTOR

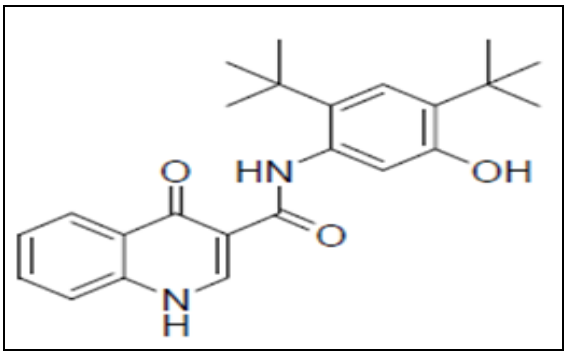

FIG. 2: STRUCTURE OF IVACAFTOR

Hence, present work focused on the development and validation of simple, rapid, robust, and economical stability indicating UPLC method. To the best of our knowledge, the anticipated method is the first UPLC method to allow simultaneous estimation of Tezacaftor and Ivacaftor in the tablet dosage form.

MATERIALS AND METHODS:

UPLC Method:

Apparatus: The separation was carried on Waters Acquity UPLC 2996 with Empower 2 software that consisted of a binary solvent manager equipped with the automatic sampler. An equity UPLC HSS C18 100 × 2.1mm 1.7µ the column was used for separation of active ingredients. Analytes were monitored with TUV detector at a wavelength of 292 nm. Ultrasonicator was used to remove dissolved gases and air bubbles in the mobile phase.

Materials: Tezacaftor and Ivacaftor standard sample were obtained as gift samples from Spectrum Labs, Hyderabad. HPLC grade water and methanol were purchased from Merck Ltd., Mumbai. Analytical grade acetonitrile and orthophosphoric acid were obtained from Rankem, Avantor Performance Material India Ltd. Marketed formulation of the combination was purchased from the local market.

Chromatographic Conditions: Separation of analytes was achieved with a mobile phase consisting of 0.1% OPA and acetonitrile at a ratio of 50: 50 delivered at a flow rate of 0.3 ml/min through column kept at 25 ºC. The volume of injection was 1 µl, and runtime was 2 min. The eluents were detected at a wavelength of 292 nm. Chromatograms of optimized method and standard were shown in **Fig. 3** and **Fig. 4**.

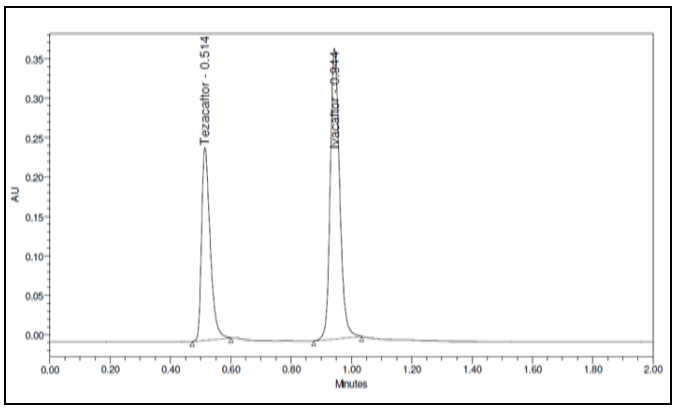

FIG. 3: CHROMATOGRAM OF OPTIMIZED METHOD

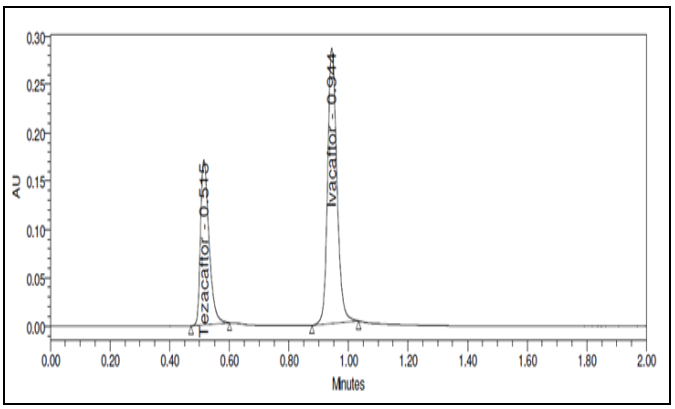

FIG. 4: CHROMATOGRAM OF STANDARD PREPARATION

Preparation of Standard Stock Solutions: Accurately weighed and transferred 25 mg of Tezacaftor and 37.5 mg of Ivacaftor working Standards into a 25 ml clean, dry volumetric flask, add a 3/4th volume of diluent, sonicated for 5 min and makeup to the final volume with diluents. (1000 ppm of Tezacaftor and 1500 ppm of Ivacaftor). 1 ml from the above two stock solutions were taken into a 10 ml volumetric flask and made up to 10 ml. (100 ppm of Tezacaftor and 150 ppm of Ivacaftor)

Preparation of Sample Stock Solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (1000 ppm of Tezacaftor and 1500 ppm of Ivacaftor). From the filtered solution, 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent. (100 ppm of Tezacaftor and 150 ppm of Ivacaftor).

Validation of the HPLC Method:

System Suitability: The developed method was validated according to ICH guidelines [10]. To check the system performance, the system suitability parameters were measured. System precision was determined on six replicate injections of standard preparations. A number of theoretical plates and asymmetry were measured [11-12]. The system suitability parameters were determined by preparing standard solutions of 100 ppm of Tezacaftor and 150 ppm of Ivacaftor and the solutions were injected six times, and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Linearity: A series of solutions are pipette out from the standard stock solutions (100 ppm of Tezacaftor and 150 ppm of Ivacaftor), and diluted with mobile phase up to 10 ml volumetric flask. The calibration graphs were plotted over 5 different linear concentrations in the range of 25-150 µg/mL for Tezacaftor and 37.5 - 225 µg/mL.

Accuracy: Accuracy is the percent of analyte recovered by assay from a known added amount. For the measurement of accuracy data from nine determinations over three concentration levels covering the specified range were determined.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as % R.S.D. for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on 3 different days, and the results documented as standard deviation and % R.S.D [13].

LOD and LOQ: The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision, and variability (ICH guideline Q2B, 2005).

Robustness: The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical condition ns like flow rate (+0.1 mL min^{-1}), and mobile phase composition (2%).

RESULTS AND DISCUSSION:

System Suitability: The retention times of Tezacaftor and Ivacaftor were found to be 0.514 and 0.944 min, respectively. Plate count and tailing factor were very satisfactory, so this method was optimized and to be validated. System suitability results were in **Table 1**.

According to the USP, the HPLC method is considered suitable when the ticagrelor of peak area <1%, tailing factor <2, and the theoretical plates >2000. All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The results of system suitability are shown in **Table 1**.

Linearity: Six linear concentrations of Tezacaftor and Ivacaftor 25-150 µg/ml, 37.5-225 µg/ml was injected in a duplicate manner. Correlation coefficient obtained was 0.999 and 0.999 for both drugs. The regression analysis is shown in **Table 2**.

TABLE 1: SYSTEM SUITABILITY

S. no.	Tezacaftor			Ivacaftor			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	0.514	3613	1.47	0.943	4661	1.18	7.9
2	0.514	3601	1.47	0.943	4653	1.18	7.9
3	0.514	3609	1.47	0.944	4666	1.18	7.9
4	0.515	3597	1.47	0.944	4683	1.18	7.9
5	0.515	3608	1.47	0.944	4663	1.18	7.9
6	0.515	3615	1.46	0.944	4680	1.18	7.9

TABLE 2: LINEARITY RESULTS

S. no.	Tezacaftor		Ivacaftor	
Inj	Conc (µg/ml)	Area	Conc (µg/ml)	Area
1	0.514	3613	0.943	4661
2	0.514	3601	0.943	4653
3	0.514	3609	0.944	4666
4	0.515	3597	0.944	4683
5	0.515	3608	0.944	4663
6	0.515	3615	0.944	4680

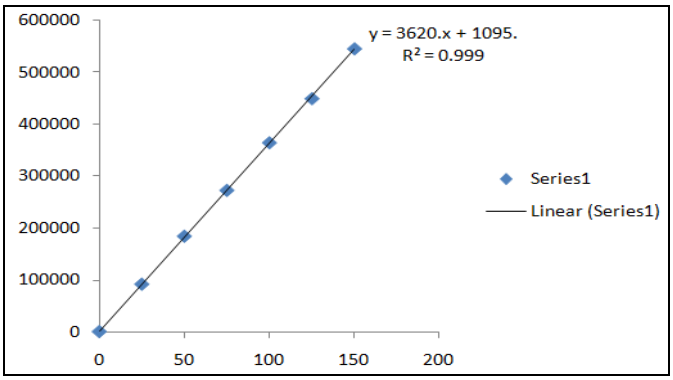

FIG. 5: CALIBRATION GRAPH OF TEZACAFTOR

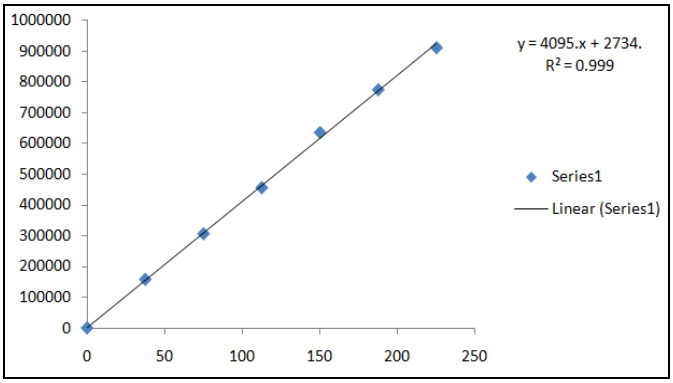

FIG. 6: CALIBRATION GRAPH OF IVACAFTOR

Accuracy: Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.97% and 99.65% for Tezacaftor and Ivacaftor respectively.

Precision: Multiple sampling from a sample stock solution was done, and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation, and % RSD were calculated for both drugs. As the limit of Precision was less than "2," the system precision was passed in this method.

TABLE 3: ACCURACY TABLE OF TEZACAFTOR

% Level	Amount Spiked(µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	50	49.73239	99.46	99.97
	50	50.1958	100.39	
	50	50.13366	100.27	
100%	100	100.8498	100.85	
	100	98.98895	98.99	
	100	99.88511	99.89	
150%	150	151.3792	100.92	
	150	148.8652	99.24	
	150	149.6297	99.75	

TABLE 4: ACCURACY TABLE OF TEZACAFTOR

% Level	Amount Spiked(µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	75	74.70	99.60	99.65
	75	74.77	99.69	
	75	74.84	99.79	
100%	150	150.0403	100.03	
	150	149.3551	99.57	
	150	148.9477	99.30	
150%	225	222.8901	99.06	
	225	223.9617	99.54	
	225	225.6147	100.27	

Limit of Detection and Limit of Quantification (LOD and LOQ): The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified.

The limit of quantitation is the lowest injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography. The quantitative limit is particularly used for the determination of impurities and degradation products. The results were shown in **Table 5**.

TABLE 5: LOD AND LOQ RESULTS OF TEZACAFTOR AND IVACAFTOR

Parameters	Tezacaftor	Ivacaftor
Limit of Quantification (µg/mL)	0.37	1.24
Limit of Detection (µg/mL)	0.12	0.41

Robustness: Robustness conditions like Flow minus (0.27 ml/min), flow plus (0.33 ml/min), mobile phase minus (55B:45A), mobile phase plus (65B:35A), temperature minus (25 ℃) and temperature plus (35 ℃) was maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected, and all the parameters were passed. % RSD was within the limit.

TABLE 6: ROBUSTNESS DATA FOR TEZACAFTOR AND IVACAFTOR

S. no.	Condition	% RSD of Tezacaftor	% RSD of Ivacaftor
1	Flow rate (-) 0.27ml/min	0.9	0.4
2	Flow rate (+) 0.33ml/min	1.0	0.2
3	Mobile phase (-) 55B:45A	1.0	0.4
4	Mobile phase (+) 65B:35A	0.6	0.3
5	Temperature (-) 25°C	1.0	0.7
6	Temperature (+) 35°C	0.8	0.8

Assay: SYMDEKO is co-packaged as a Tezacaftor / Ivacaftor fixed-dose combination tablet and an Ivacaftor tablet. Both tablets are for oral administration. The Tezacaftor/Ivacaftor fixed-dose combination tablet is available as a yellow, capsule-shaped, film-coated tablet containing 100 mg of Tezacaftor, 150 mg of Ivacaftor, Average % Assay for Tezacaftor and Ivacaftor obtained was 100.02% and 99.85% and shown in **Table 7**.

TABLE 7: ASSAY DATA OF TEZACAFTOR AND IVACAFTOR

S. no	Standard Area Tezacaftor	Standard Area Ivacaftor	Sample area Tezacaftor	Sample area Ivacaftor	% Assay Tezacaftor	% Assay Ivacaftor
1	363359	623398	362161	620434	100.11	99.61
2	360892	627879	362358	618893	100.16	99.37
3	359772	625100	361457	618626	99.91	99.32
4	359217	618517	360901	623794	99.76	100.15
5	365846	618239	361107	623766	99.82	100.15
6	359496	616411	362993	625749	100.34	100.47
Avg	361045	621591	361830	621877	100.02	99.85
Stdev	2641.9	4530.7	808.2	2959.4	0.223	0.48
% RSD	0.7	0.7	0.2	0.5	0.2	0.5

Forced Degradation Studies: Forced degradation studies were conducted to know the stability of the method. The degradation studies were carried out by applying various stress conditions for the

product like acid stress, base stress, UV stress, humidity stress, thermal stress, and oxide stress. Degradation peaks were observed only in acid stress, and peroxide stress and all degradation peaks were well resolved from analyte peaks. The results of forced degradation studies were shown in **Table 8**.

TABLE 8: RESULTS OF FORCED DEGRADATION STUDIES

Type of degradation	Tezacaftor			Ivacaftor		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	330357	91.22	8.78	592590	95.14	4.86
Base	340688	94.07	5.93	603273	96.86	3.14
Peroxide	349985	96.64	3.36	603807	96.94	3.06
Thermal	352397	97.31	2.69	604201	97.01	2.99
UV	358327	98.94	1.06	615824	98.87	1.13
Water	360579	99.56	0.44	618309	99.27	0.73

CONCLUSION: The developed UPLC analytical method provides an eco-friendly, reliable, reproducible, simple, rapid, sensitive, accurate, precise and specific assay method for the simultaneous estimation of Tezacaftor and Ivacaftor in pharmaceutical formulations. Degradation studies reveal that the developed method was stability indicating. Hence, the proposed method can be conveniently used for the routine analysis of Tezacaftor and Ivacaftor in pure and pharmaceutical dosage forms.

ACKNOWLEDGEMENT: The authors are grateful to University College of Technology, Osmania University for providing necessary facilities to carry out the above research work.

CONFLICT OF INTEREST: There is no conflict of interest declared by authors.

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How to cite this article:
Shyamala and Ashok D: A novel stability indicating UPLC method for the estimation of Tezacaftor and Ivacaftor in tablet dosage form. Int J Pharm Sci & Res 2019; 10(11): 4968-73. doi: 10.13040/IJPSR.0975-8232.10(11).4968-73.