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A New Stability-Indicating RP-UPLC Method Development and Validation for the Simultaneous Estimation of Ivacaftor and Tezacaftor in Pharmaceutical Dosage Form

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Abstract
A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivacaftor and Tezacaftor in tablet dosage form. Chromatogram was run through CHS C18 (100 x 2.1 mm, 1.7µ) and the mobile phase containing water: acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 0.3 ml/min. The temperature was maintained at 30 °C throughout the method. Optimized wavelength selected was 292.0 nm. Retention time of Ivacaftor and Tezacaftor were found to be 0.620 min and 1.155 min. %RSD of the Ivacaftor and Tezacaftor were and found to be 0.5 and 0.5 respectively. %Recovery was obtained as 99.52% and 100.61% for Ivacaftor and Tezacaftor respectively. LOD, LOQ values obtained from regression equations of Ivacaftor and Tezacaftor were 0.30, 0.10 µg/ml and 0.91, 0.31 µg/ml respectively. Regression equation of Ivacaftor is y = 5052.x + 958.8, and y = 5012.x + 245.4 of Tezacaftor. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

Keywords
Ivacaftor, Tezacaftor, RP-UPLC, stability indicating, method development, validation.

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1. INTRODUCTION
Ivacaftor is a cystic fibrosis transmembrane conductance regulator (CFTR) modulator which was first official by FDA moreover it shows the significant improvement in the lung function of cystic fibrosis (CF) by clinical evidence[1]. It is producing enhanced CFTR channel release possibility to enhance chloride influx works as a CFTR potentiator[2-4]. Ivacaftor is noticeably metabolized by means of cytochrome P450 enzymes, initially to dynamic metabolite

hydroxymethyl- Ivacaftor (M1) and an inactive form Ivacaftor-carboxylate (M6) [5-8] Ivacaftor is chemically named as *N-(2, 4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide*[9-13] and the structure was shown in the Fig.1. Tezacaftor helps travel the CFTR protein to the exact site on the cell surface, and is intended to take care of people with the F508del transmutation[14]. Tezacaftor is an examining CFTR corrector that, in mixture with ivacaftor, has been revealed to develop lung function and reduce sweat chloride concentrations within a phase 2 clinical examination concerning patients who were homozygous intended for the Phe508del *CFTR* transformation and patients who were heterozygous designed for the Phe508del *CFTR* transmutation plus the G551D *CFTR* transmutation[15]. Tezacaftor chemically termed as *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*[14-15] and the structure was shown in the Fig.2.

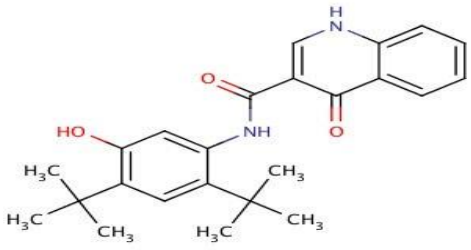


Fig.1: Structure of Ivacaftor

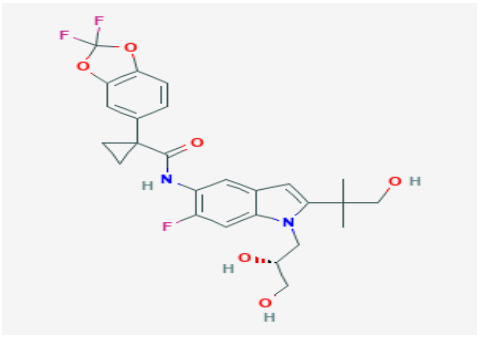


Fig.2: Structure of Tezacaftor

2. MATERIALS AND METHODS

2.1 Instrumentation

The analysis was eluted on an Acquity UPLC SYSTEM used was of column CHS C18 (100X2.1mm) 1.7mm with quaternary pumps, ACQUITY TUV detector and Auto sampler integrated with the software Empower 2.0. It was equipped with UV-VIS spectrophotometer PG Instruments T60 having particular bandwidth of 2 mm and 10 mm and coordinated quartz cells incorporated with the software UV win 6. It was used for measuring absorbencies of ivacaftor and tezacaftor solutions.

2.2 Materials

UPLC grade acetonitrile, water was secured from Ranbaxy, India, and Distilled water, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, and Ortho-phosphoric acid was secured from Ranbaxy laboratories Ltd, India were used in the current method and supported by the literature. The drug samples were kindly issued by Spectrum Pharmaceuticals Pvt Ltd, Kukutpally, Hyderabad, India, and the formulation samples and high purity distilled water were used. The preparation of two drugs was Symdeko tablets labeled claim was 150 mg of mg of ivacaftor and 100 mg of tezacaftor.

A few analytical methods have been reported for ivacaftor and tezacaftor individually or with some other combination; some of them were being development of an RP-HPLC method for analysis of ivacaftor in degradation products[16-18], LC-MS method[12], new process development and validation for instantaneous evaluation of tezacaftor and ivacaftor in drug dosage form with UV spectrophotometer [19].

There is no official method reported for this combination in the UPLC method development and validation so far. The most important plan of this method was to determine and validate, in assimilation with ICH (International Conference on Harmonization rules)[20-23]. A precise, reasonable, and reproducible method for quantitative analysis of ivacaftor and tezacaftor in the tablet dosage forms as well as bulk drug. It was thought suitable to develop specific, correct, simple method of UPLC for instantaneous evaluation of ivacaftor and tezacaftor.

2.3 Chromatographic conditions

A mobile phase consisting of buffer and acetonitrile was taken with the ratio of 50:50A V/V and the pH were adjusted to 3 with water and methanol with the ratio of 50:50 V/V made use as diluents intended for preparing the operational solutions of drug. The separation was achieved with the elution method**.** The flow rate was maintained 0.3 ml/min and the total injection volume was taken 1.0 ml every time. The eluent was monitored by the tunable UV detector (TUV) from 190 to 700 nm, and chromatograms be extracted at the wavelengths of 292 nm. The total run time was 2 min, and all establishments were performed at 30 °C.

2.4 Standard solutions

2.4.1Diluent: The diluents Acetonitrile and Water in use in the ratio of 50:50 were selected based on the solubility of drug samples.

2.4.2 Stock solutions preparation:

Exactly weighed 15 mg of Ivacaftor and 10 mg of Tezacaftor was taken and transferred to 25 ml standard flask. And three fourth of diluents were mixed to these flasks. The solution was sonicated for 10 minutes. The prepared solutions in flask labeled as Standard stock solution. The solution was becoming 600 µg/ml of Ivacaftor and 400 µg/ml of Tezacaftor. The regular operational solutions (100% solution) were prepared by using 1ml from both stock solutions were pipetted out into a 10 ml volumetric flask and made up to the mark with diluent. The solution was made up to 60 µg/ml of ivacaftor and 40 µg/ml of tezacaftor.

2.4.3 Assay of sample preparation

10 tablets were weighed by using electronic balance (Denver) and the standard weight of tablet was computed, then the weight of 1 tablet equivalent was placed into a 100 ml standard flask. The diluents of 50 ml were mixed and sonicated for 25 min, auxiliary the solution was made up to the mark with diluent and filtered by UPLC filters. The dilution became 1500µg/ml of ivacaftor and 1000µg/ml of tezacaftor. The sample preparation was done taking 0.4 ml of filtered stock solution was taken into 10 ml volumetric flask and made up to the mark with diluent. The dilution of samples was 60 µg/ml of ivacaftor and 40 µg/ml of tezacaftor. These specified concentrations were used for general procedure and recovered concentrations were calculated from the consequent calibration graphs. For regular addition assay, test solutions were impaled with aliquots of regular solutions of the three compounds to gain total concentrations contained by the earlier specified ranges then considered as underneath common process. Recovered concentrations were deliberated by assimilating the analyte response with the growth response achieved after addition of the standard.

2.5 Method validation procedure

The current process was effectively validated as per ICH guidelines[20-23]. The different validation parameters which were performed are the following: system suitability, linearity, precision, accuracy, specificity, and limit of detection (LOD), limit of quantification (LOQ), robustness, and degradation studies.

2.6 Degradation studies

2.6.1 Oxidation:

To the 1 ml of stock solution of ivacaftor and tezacaftor The 1 ml of 20% hydrogen peroxide (H_2O_2) was mixed separately. The obtained solutions set aside for 30 min under a temperature of 60 ^{0}C. For UPLC study, the consequential solution was diluted to acquire 60 µg/ml and 40 µg/ml solution and chromatograms were recorded by injecting 1 µl of resultant solution to analyze the steadiness of sample.

2.6.2Acid Degradation Studies:

1 ml of 2N Hydrochloric acid was added to the 1 ml stock solution of ivacaftor and tezacaftor. The obtained solution was refluxed for 30mins at 60 ^{0}C. The solution was converted to 60µg/ml and 40µg/ml by using diluents. The chromatograms were achieved to analyze stability f samples by injecting 1 µl solution in to the system.

2.6.3 Alkali Degradation Studies:

1 ml of 2N sodium hydroxide was added to the 1 ml stock solution of ivacaftor and tezacaftor. The obtained solution was refluxed for 30 mins at 60 ^{0}C. The solution was converted to 60µg/ml and 40µg/ml by using diluents. The chromatograms were recorded to analyze stability of samples by injecting 1 µl solution into the system.

2.6.4 Dry Heat Degradation Studies:

The ivacaftor and tezacaftor solution was kept in oven at 105°C for 1 hour to study dry heat degradation. For UPLC study, the solution was converted to 60µg/ml and 40µg/ml by using diluents. The chromatograms were recorded to analyze stability f samples by injecting 1 µl solution in to the system.

2.6.5 Photo Stability studies:

The ivacaftor and tezacaftor solutions of 60 µg/ml and 40 µg/ml were kept in UV Chamber for 1 day or 200 Watt hours/m^2 to study the photochemical steadiness of the drug samples. The chromatograms were acquired to analyze stability f samples by injecting 1 µl solution in to the system.

2.6.6 Neutral Degradation Studies:

Natural degradation studies were conceded by refluxing the ivacaftor and tezacaftor in water for 1 hour at a temperature of 60 ºC. For UPLC study, the solution was converted to 60µg/ml and 40µg/ml by using diluents. The chromatograms were noted to analyze neutral stability of samples by injecting 1 µl solution in to the system.

3. RESULTS AND DISCUSSION

A simple, speedy and a perfect method was developed and validated for the drug samples of

ivacaftor and tezacaftor. There is no official method for this combination so far in the Rp-UPLC method development and validation. However, few methods have been reported in either of one or two in this combination with some other drugs.

On comparison with literature, the mobile was used buffer-acetonitrile (30:70 % v/v) [16] a mixture of 45 volumes of acetonitrile and 55 volumes of mixed phosphate buffer [18] Acetonitrile: 0.25 Mm Potassium dihydrogen orthophosphate buffers [24]. The retention times and runtimes were shown in above methods are very high. RP-UPLC method is the most powerful than other RP-HPLC, LC-MS and UV spectroscopy methods [25-26].

In the proposed method a simple mobile phase consisting of buffer and acetonitrile was used which elute the ivacaftor and tezacaftor with lower retention time. The retention times 0.617 min and 1.146 for ivacaftor and tezacaftor respectively. The calibration curve was linear over the concentration range of 15-90 ppm and 10-60 respectively. The LOD values were 0.30, and 0.10 and LOQ values were found to be 0.91and 0.31for ivacaftor and tezacaftor respectively. The high percentage of revival and low percentage coefficient of difference authenticate the appropriateness of the method and the forced degradation studies shows that the developed method was stability indicating. Hence it was completed that the RP-UPLC method developed was highly suitable for usual study and all the parameters result data was shown in method validation systematically.

3.1 Method Development

Six trials were conducted for the method development and the best peaks with least fronting factor was elevated for ivacaftor and tezacaftor with RT = 0.617 min, RT = 1.146 min. accordingly. The resultant chromatogram is revealed in the **Fig. 3&4**.

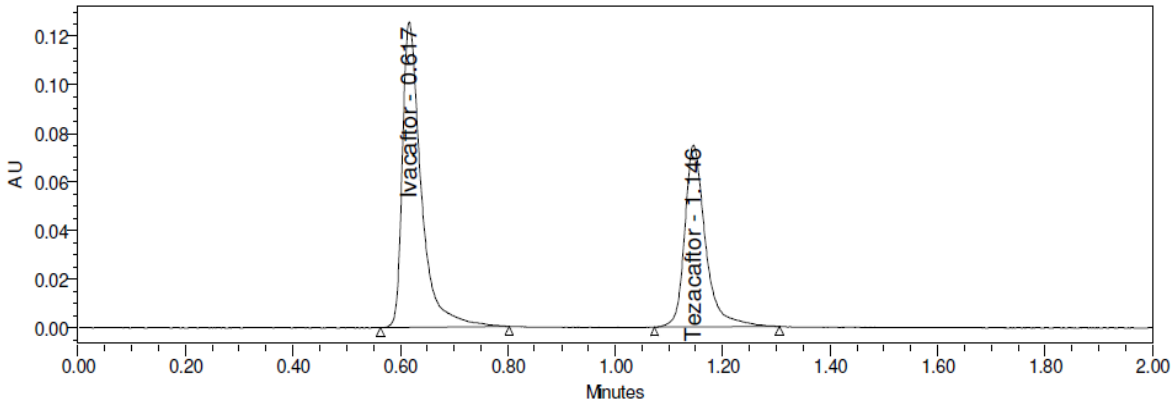


Fig. 3: Sample chromatogram of ivacaftor and tezacaftor

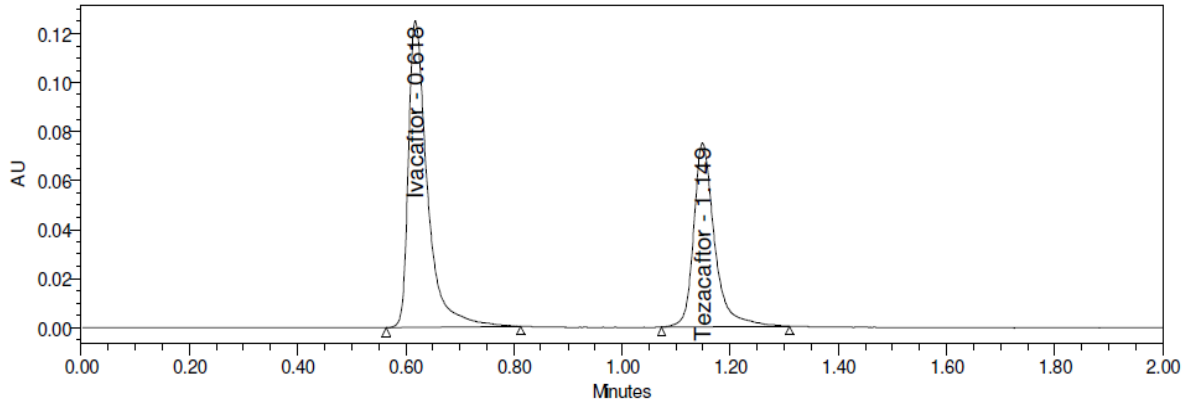


Fig. 4: Standard chromatogram of ivacaftor and tezacaftor

3.2 Method validation

The method was validated as per ICH guidelines [20-23]. The different validation parameters which were determined are the following: system suitability test, precision, accuracy, linearity and specificity, limit of detection, limit of quantification, robustness, degradation studies and the stability indicating capability.

3.3 System suitability test

Six repeat injections of standard solution of ivacaftor and tezacaftor were injected and the chromatograms be recorded. The system was appropriate for examination if the % relative standard deviation (%RSD) of area counts in six repeat injections must be not more than 2.0%. USP tailing factor for ivacaftor and tezacaftor peak should be not more than 2.0. The USP resolution factor between the peaks corresponding to ivacaftor and tezacaftor should be more than 2.0. The results are revealed in **Table 1**.

Table-1: System suitability parameters of ivacaftor and tezacaftor

Parameters	Ivacaftor	Tezacaftor
Tailing Factor	1.77	1.35
Theoretical plates	4727	4822
USP Resolution	--	8.2
LOD(µg/ml)	0.30	0.10
LOQ(µg/ml)	0.91	0.31

3.4 Precision

The standard ivacaftor and tezacaftor solutions were injected for six times and measured the area for all six injections in UPLC. The % RSD for the area of six repeat injections was established to be within the specific limits. The data was presented in the **Table 2**.

Acceptance Criteria: The % RSD should not be more than 2%.

TABLE 2: Precision study of ivacaftor and tezacaftor

S.No.	Ivacaftor			Tezacaftor		
	Peak area	%Assay	Day_day Precision	Peak area	%Assay	Day_day Precision
1	305751	99.63	289043	203309	100.18	202573
2	306025	99.71	290150	204840	100.94	204527
3	307641	100.24	285821	204390	100.72	206378
4	307422	100.17	289775	202256	99.66	205674
5	310072	101.03	289357	201842	99.46	204052
6	305668	99.60	288023	202704	99.89	201777
AVG	307097	100.06	288695	203224	100.14	204164
SD	1688.5	0.55	1584.6	1191.3	0.6	1763.8
%RSD	0.5	0.5	0.5	0.6	0.6	0.9

3.5 Accuracy

Accuracy of the readings was computed by % recovery of six different concentrations of ivacaftor and tezacaftor at 50%, 100% and 150% and also standard addition technique was carried out for same samples. The results acquired including the means of the recovery and standard deviations were displayed in **Table 3**.

Acceptance Criteria: The % Recovery for ivacaftor and tezacaftor at each stage should be between 99 to 101%.

Table 3: Accuracy data of ivacaftor and tezacaftor

Sample	ivacaftor			tezacaftor		
%Concentration	50%	100%	150%	50%	100%	150%
Trail-I	99.77	99.38	100.07	100.86	101.87	100.78
Trail-II	99.80	98.34	99.35	100.25	100.90	100.01
Trail-III	100.20	99.28	99.51	100.79	101.04	98.97
AVG (%Recovery)	99.52			100.61		
SD	0.55			0.80		
%RSD	0.55			0.8		

3.5.1 Recovery studies

To estimate the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and reference drugs were added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits.

3.6 Linearity and Calibration Curve:

Working dilutions of ivacaftor and tezacaftor in the range of 15 to 90 and 10 - 60% were prepared by considering appropriate aliquots of functioning standard solutions of drugs in various 10 ml volumetric flask and diluting up to the mark with mobile phase. 1 µl amount of each dilution was send into the system at a flow rate of 0.3ml/min. The drug in elute was monitored at 292 nm and the resultant chromatograms were recorded. From these, the mean peak areas were computed and shown in the **Table 4**. A plot of concentration vs. peak areas was constructed and shown in the **Fig. 5** and **6** for ivacaftor and tezacaftor respectively.

The regression of the plot was calculated by least square regression method. The slope and intercept value for calibration curve for ivacaftor and tezacaftor was y = 5070.x + 1282. R^2 = 0.999 and y=5012.x+245.4 (R2=0.999) respectively.

Table-4: Linearity means peak area values

S.No	Concentration of ivacaftor (µg/ml)	Response	Concentration of tezacaftor (µg/ml)	Response
1	0	0	0	0
2	15	78014	10	49561
3	30	154896	20	101725
4	45	232776	30	151631
5	60	302737	40	199479
6	75	375523	50	250795
7	90	462260	60	301660

Figure-5: Linearity graph ivacaftor

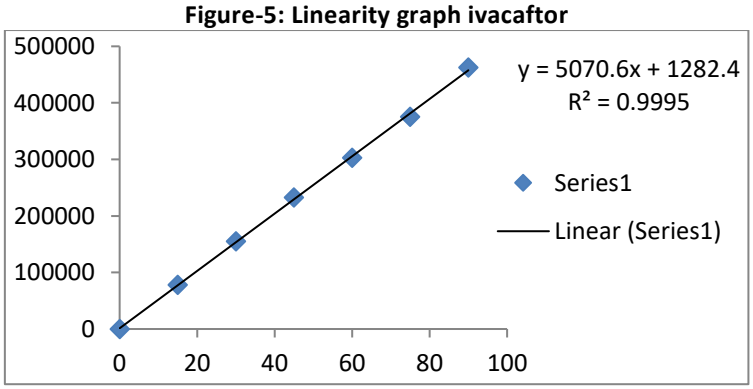
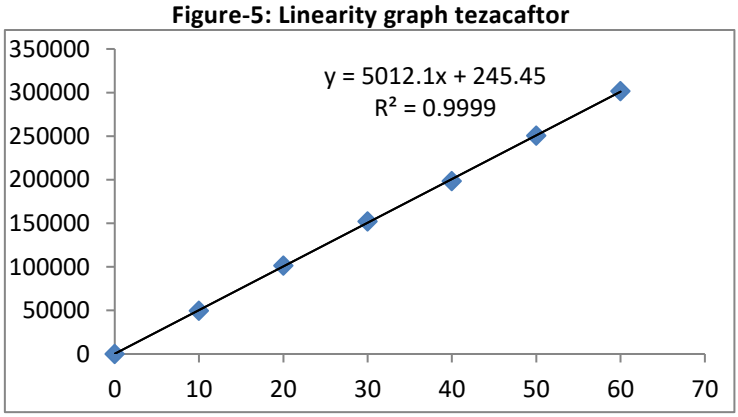


Figure-5: Linearity graph tezacaftor



X-Axis = Concentration; Y-Axis = Peak area

3.7 Specificity

The specificity of the RP-UPLC method is furnished, where complete separations of ivacaftor and tezacaftor were distinguished in presence of other inert excipients used in tablets. In addition, there was no deterrence at the retention time of in the chromatogram of placebo solution. In the case of peak purity analysis with PDA, purity gradient was always not greater than purity threshold for the analytes. This shown that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were listed in the table 5.

Table-:5 Specificity studies of ivacaftor and tezacaftor

S.No.	Name	No. of Injections	ivacaftor Area	tezacaftor Area
1	Blank	1	-	-
2	Placebo	1	-	-
3	Standard	1	307520	203495
4	Sample	1	299926	198584

3.8 Limit of detection and limit of quantification

Limit of Detection (LOD) is the least concentration of an analyte in a sample that can be identified but not quantified. LOD is indicated as a concentration at a précised signal to noise ratio. The LOD will depend on the process of examination along with type of instrument. In the chromatography, detection limit is the injected quantity that consequences in a peak with a height at least thrice or twice as high as baseline noise level. LOD was computed by using formula LOD=3.3(SDS)

The LOD was found to be 0.30 and 0.10 for ivacaftor and tezacaftor respectively.

Limit of quantification (LOQ) is least concentration of analyte in a sample that can be estimated with tolerable precision, accuracy and reliability by a specified method under affirmed experimental conditions. LOQ is uttered as a concentration at a specified signal to noise ratio. In the chromatography, limit of quantification is the injected amount that consequences in a peak with a height, ten times as high as foundation line noise level. LOQ is calculated by using the formula LOQ=10(SDS)

The LOQ was originated to be 0.91 and 0.31 for ivacaftor and tezacaftor respectively.

3.9 Robustness

Robustness is denoted by making speculate changes in the chromatographic conditions like change in temperature, mobile phase composition and flow rate were assessed for the impact on the present method. It was founded from the chromatograms that the results were not more than the limits. This represents that the method developed is robust and shown in the Table 6.

Table-6: Robustness study of ivacaftor and tezacaftor

Parameter		ivacaftor	Tezacaftor
Temperature±5^{0}C	25^{0}C	303632	200568
	35^0 C	305907	200720
Flow rate±0.1ml	0.2 ml	305784	247311
	0.4 ml	304761	181391
Mobile Phase change ±5 pH	45:55	304644	203305
	55:45	307710	194305

3.10 Degradation studies

The stress studies were conceded out to ensure the effective separation of ivacaftor and tezacaftor in the present study from degradation products. The degradation was shown by reducing the peak areas of the drug samples with similar drug molecules of degraded peak areas. The percentage assay of degradation was calculated from the peak area obtained in degradation conditions and it was compared with assay of non-degraded conditions. The percentage assay degradation in both acidic and alkali conditions was found to be within the limits. Oxidative degradation studies were conducted by using peroxide solution and the results showed that there were no degradation products formed. The drug solutions were kept in oven at 105 °C for 6 h for thermal degradation studies and then injected into HPLC system and photo stress testing was conceded

out by keeping the drug solutions in UV chamber for 7 days. The purity of angle is established to be less than that of purity of threshold in all the circumstances which indicates that the developed technique was stability indicating. The stress studies were performed without planning to recognize the degradation products but only to show that they are not interfering with active molecules if any present. The data of stress studies are given away in table 7.

Table 7: Degradation studies of ivacaftor and tezacaftor

Sample Name	Total purity	ivacaftor			tezacaftor		
		% of Purity	%of Degradation	Purity of peak area	%of Purity	% of Degradation	Purity of peak area
cid	100	94.91	5.09	306646	94.25	5.75	203191
Base	100	95.55	4.45	307520	95.16	4.84	203195
Peroxide	100	96.72	3.28	305257	96.12	3.88	202029
Thermal	100	97.04	2.96	306623	97.51	2.49	201963
Uv	100	98.14	1.86	306157	98.04	1.96	202517
Water	100	98.14	1.86	305523	99.17	0.83	202291

4. CONCLUSIONS

The current study describes new and simple, reliable, economic elution RP-HPLC-PDA method for the simultaneous estimation of ivacaftor and tezacaftor. The forced degradation studies were conducted for the three drugs by using several degradation conditions like oxidation, acidic, alkali, thermal, and photolytic conditions and proposed method was effectively employed from the resolution of employed samples peaks. To the best of our knowledge, no such detailed and stability indicating method has been presented for the assay of this triplicate drug mixture. The developed method finished use of UPLC as a tool for peak integrity and purity confirmation. Therefore, the proposed study method can be used for quantification ivacaftor and tezacaftor in bulk and pharmaceutical dosage form. Finally, this method was carefully validated; as a result, it can be suggested for routine analysis and for testing quality through stability studies of the drugs.

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