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*Research Article*

# A NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ANALYSIS OF IVACAFTOR AND TEZACAFTOR IN PURE AND ITS PHARMACEUTICAL FORMULATIONS

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**ABSTRACT**

A novel stability-indicating RP-HPLC method for the simultaneous analysis of Ivacaftor and Tezacaftor in pure and its pharmaceutical formulations. Chromatographic separations were achieved in Shemadzu, Empower 2 software, module of separation is 2695, UV detector equipped with Kromisil $C_{18}$ (150 x 4.6 mm, 5 µm) using mobile phase is water: acetonitrile, (50:50, v/v), detection wavelength at 292 nm. The $R_t$ of Ivacaftor is 2.089 min and $R_t$ of Tezacaftor is 2.434 min was found. The linearity ranges was 15-90 µg/ml and 10-60 µg/ml of Ivacaftor and Tezacaftor, respectively. All the validation parameters must be reached with acceptable limits. The proposed method was simultaneous analysis of Ivacaftor and Tezacaftor successfully applied in quality control labs.

**Keywords**: Ivacaftor, Tezacaftor, Analysis, Kromisil and Validation.

## INTRODCTION

Ivacaftor chemical name as N-(2,4-ditert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxmide and category is CF TR potentiator. Ivacaftor was used for the treatment of cystic fibrosis and it will alter the activity of the CFTR channels. Tezacaftor chemical name is 1-(2, 2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl}cyclopropane-1-carboxamide) and category was not available. Tezacaftor was used for the treatment of cystic fibrosis in some cases. Literature review reveals that few methods have been reported for the estimation of Ivacaftor and Tezacaftor with other combined drugs using UV[1-3], HPLC[4-8]. So far, to our present knowledge, no method is reported for the stability-indicating RP-HPLC method for the simultaneous analysis of Ivacaftor and Tezacaftor. Hence, a new sensitive stability-indicating and efficient RP-HPLC for the simultaneous analysis of Ivacaftor and Tezacaftor in pharmaceutical formulations.

## MATERIALS AND METHODS

### INSTRUMENTATION

To develop a new stability-indicating RP-HPLC method for simultaneous analysis of Ivacaftor and Tazacaftor using Waters HPLC system on Kromisil $C_{18}$ column (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000 UV detector. A 10 µL Rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower2 software.

### CHEMICALS AND SOLVENTS

Ivacaftor and Tezacaftor were provided as gift sample by Spectrum Labs, Hyderabad, India. All the chemicals methanol, acetonitrile, water HPLC grade and hydrochloric acid, sodium hydroxide were of AR grade were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Commercial tablets of Ivacaftor and Tezacaftor were purchased from local market.

**Mobile phase preparation**
500 mL of water mixed with 500 mL of acetonitrile was used as a mobile phase. Sonicated the solution for 10 min and filtered through 0.45µm of membrane filter. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum.

**Standard preparation**
15 mg of Ivacaftor and 10 mg of Tazacaftor were accurately weighed, transferred to 100 mL volumetric flask and is dissolved in 70 mL of the mobile phase. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.25 µm filter and the volume is made up to 100 mL with mobile phase to get a concentration of 1 mg/mL stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

**Sample preparation**
20 commercial tablets of Ivacaftor and Tezacaftor were finely powdered and the powder equivalent to 150 mg of Ivacaftor and 100 mg of Tezacaftor were accurately weighed and transferred to 100 mL volumetric flask and dissolved in 70 mL of mobile phase. The above solution was subjected to sonication for 15 min. After getting clear solution it is filtered through 0.25 µm filter and the solution is made up to 100 mL with mobile phase resulting in preparation of 1 mg/mL solution. This is further diluted so as to obtain required concentration of Ivacaftor and Tezacaftor pharmaceutical dosage form.

**Methodology**
The RP-HPLC system was stabilized for thirty minutes by passing mobile phase, detector was set at 292 nm, flow rate of 1.0 mL/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Six replicates of each standard solutions 15, 30, 45, 60, 75, 90 of Ivacaftor and 10, 20, 30, 40, 50, 60 of Tezacaftor were injected. Calibration graph was plotted by concentration of Ivacaftor and Tezacaftor on X-axis and peak area on Y-axis and linearity curves were shown in Figure 5 and Figure.6.

**Method validation**
**Linearity**
Six standard working solutions of Ivacaftor and Tezacaftor in the concentration of 15-90 µg/ml for Ivacaftor and 10-60 µg/ml for Tezacaftor were prepared. The samples were injected in to chromatograph under selected conditions and constrict the calibration plot by taking against mean peak on Y-axis and concentration on X-axis.

**Precision**
To conducting the intra-day and inter-day analysis for check repeatability levels of Ivacaftor and Tazacaftor on the same day and different days. The chromatograms were interpreted.

**Accuracy**
To evaluate accuracy, the sample spiked with 50%, 100% and 150% concentration levels with test samples.

**Specificity**
To check the interfering of the analytes in original sample by the placebo administered in to the system.

**System suitability parameters**
The system suitability parameters like asymmetric or tailing, retention time, number of theoretical plates and resolution.

**Assay**
The assay performing the concentration of Ivacaftor and Tezacaftor result were comparing the to the original results.

**Stability-indicating studies**
Stability-indicating studies were formed as per ICH rules like acidic, basic, peroxide, thermal, photo and neutral degradation. To investigate the degradation extent and interference of peaks. 1 ml of Ivacaftor and Tezacaftor sample take into a 10 ml of volumetric flask. Add 1 ml of 0.1N Hcl to the flask reflex at $60^0$C for 30 min. The reflex solution was neutralised by the addition of 1 ml of 0.1N NaoH and make up with final volume. The resultant solution was injected into HPLC system. Similar procedure was followed by remaining studies. 1 ml of 0.1N NaoH was used for basic studies, 20% $H_2O_2$ for peroxide studies. For thermal studies, the sample put under reflex at $105^0$C for 6 hours. For UV studies sample was exposed to sun light for 7 days and neutral studies done by refluxing the sample at $60^0$C for 6 hrs. The final concentration was adjusted to attain 60 µg/ml for Ivacaftor and 40 µg/ml for Tezacaftor.

**RESULTS AND DISCUSSION**
**Mobile phase optimization**
Different trails were done to optimise the chromatographic condition for the estimation of Ivacaftor and Tezacaftor with short run time. Different combinations of mobile phase were tried like buffer: methanol, water: methanol and acetonitrile: buffer. Finally the mobile phase

with water and acetonitrile in the ration 50:50,v/v was fixed at floe rate 0.7 ml/min. Blank chromatogram was illustrated in Figure no.1, no interfering and extra peaks was observed, it indicates the specificity of the method. The detection wavelength at 292 nm was fixed. The $R_t$ of Ivacaftor and Tezacaftor were observed at 2.089 min and 2.434 min shown in Figre.2.

**Validation**

The Ivacaftor linearity range was found to be 15-90 µg/ml with egression equation y=19854x+12847 and correlation coefficient is 0.9994 and Tezacaftor linearity range was found to 10-60 µg/ml with egression equation y=19269x+3042.2 and correlation coefficient is 0.9992 related results furnished in Table.2. The precision of the method was investigated and corresponding relative standards deviations were calculated with reached the acceptable limits. The precision results displayed in Table .3. From accuracy study obtained satisfactory recovery range of 99.62-101.55% for Ivacaftor and 99.48-100.59 for Tezacaftor, results were enclosed in Table.4. The LOD and LOQ of Ivacaftor and Tezacaftor were found to be 0.62 µg/ml and 0.34 µg/ml, 1.88 and 1.02 µg/ml, respectively. The system suitability parameter furnished Table.5.

**Assay**

The assay was performed for the determination of Marketed sample and results enclosed in Table.6.

**Stability-indicating studies**

During present stability indicating studies acid degradation sample gave 1 degradent for Ivacaftor was observed at 1.844 min; and 1 degradent for Tezacaftor was observed at 3.001; basic sample gave 1 degradant for Ivacaftor at 1.861 min; and no extra peak observed. Peroxide sample not produced any extra peak of Ivacaftor and Tezacaftor; Thermal sample not gave any extra peaks of both drugs Ivacaftor and Tezacaftor, UV sample not gave any extra peaks of Ivacaftor and Tezacaftor; similarly neutral samples not gave extra peaks of both analytes. The stability indicating data of Ivacaftor and Tezacaftor was present in Table.7

**CONCLUSION**

The present method is more economical, specific, accurate, rapid and precise was found and to separate the degradatnts from samples successfully indicates the stability nature of the method. The validation parameters were found within the limits that indicate the method was suitable. The results of assay founds with in labelled amount. Hence the present method adapt for general estimation of Ivacaftor and Tezacaftor in quality control labs.

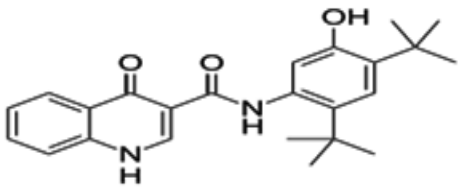


**Fig. 1: Chemical structure of Ivacaftor**

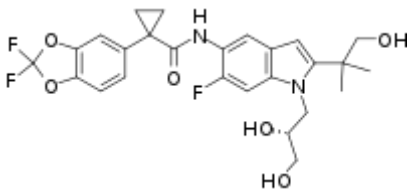


**Fig. 2: Chemical structure of Tezacaftor**

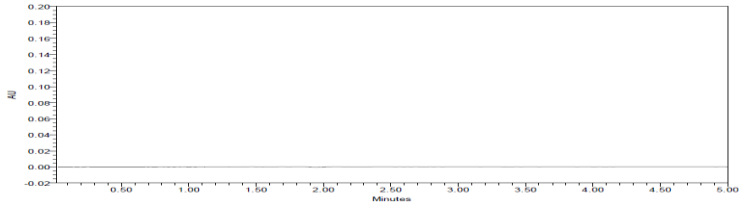

**Fig. 3: Blank chromatogram of Ivacaftor and Tezacaftor**

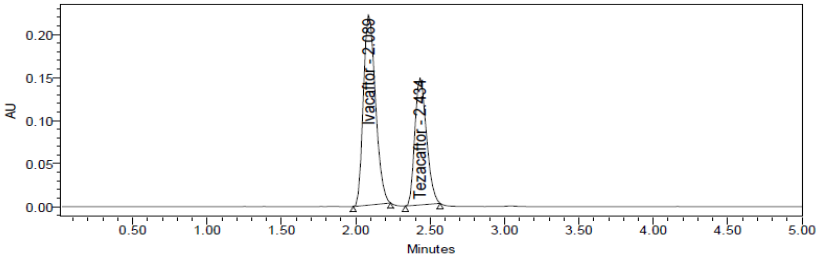


**Fig. 4: A typical chromatogram of Ivacaftor and Tezacaftor**

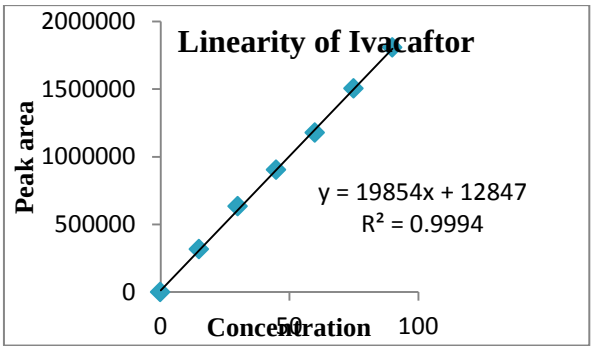


**Fig. 5: Linearity of Ivacaftor**

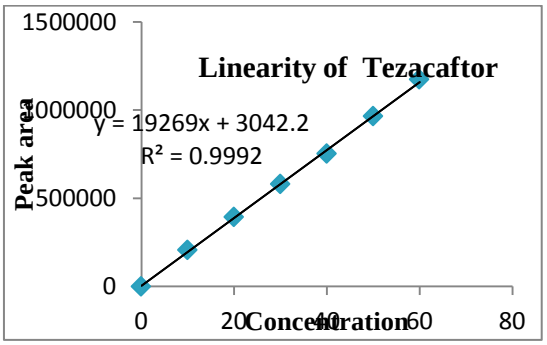


**Fig. 6: Linearity plot of Tezacaftor**

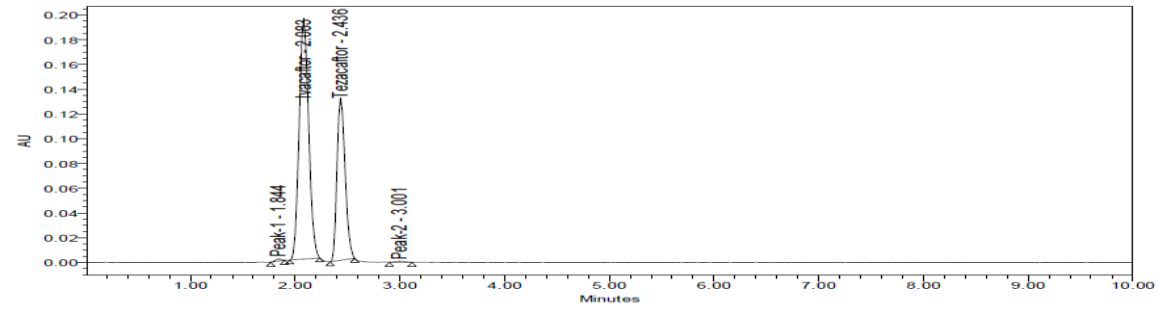


**Fig. 7: Chromatogram of Ivacaftor and Tezacaftor under acidic conditions**

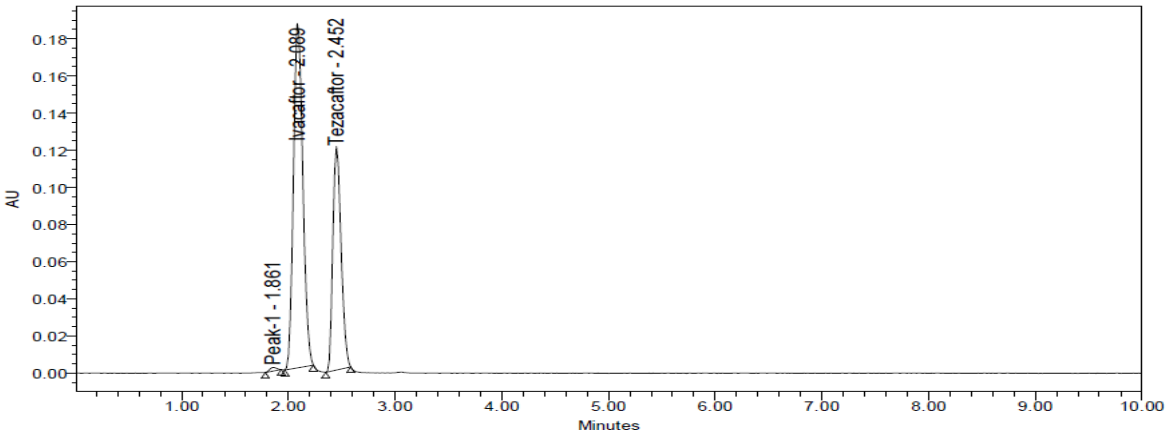


**Fig. 8: Chromatogram of Ivacaftor and Tezacaftor under basic conditions**

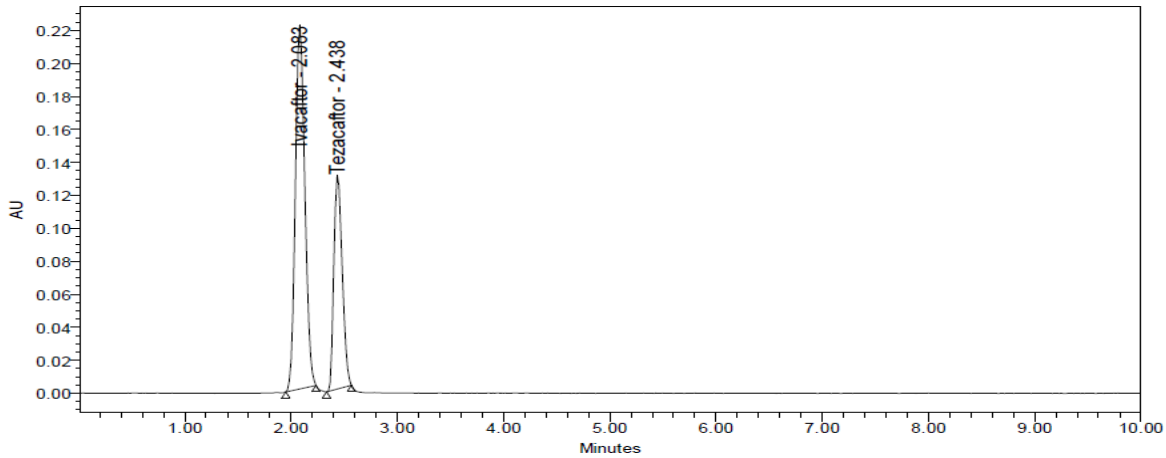


**Fig. 9: Chromatogram of Ivacaftor and Tezacaftor under peroxide conditions**

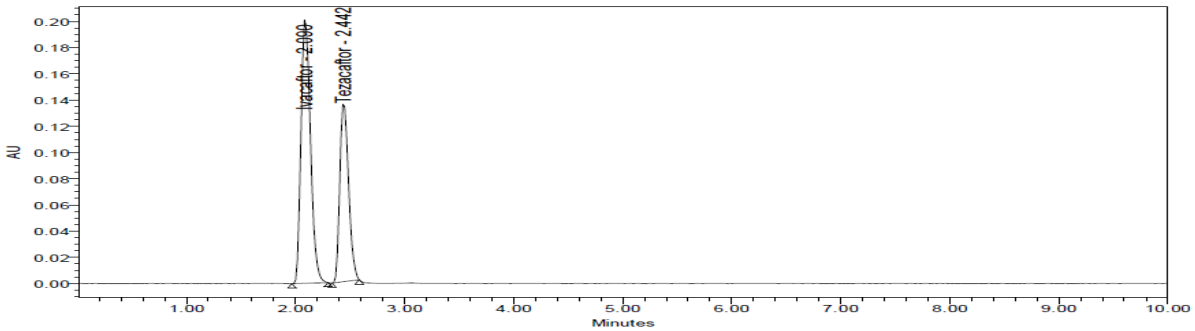


**Fig. 10: Chromatogram of Ivacaftor and Tezacaftor under thermal conditions**

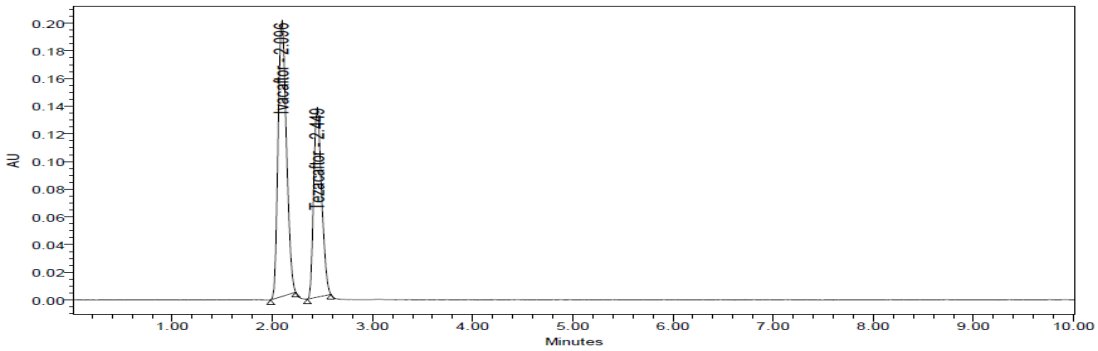


**Fig. 11: Chromatogram of Ivacaftor and Tezacaftor under UV conditions**

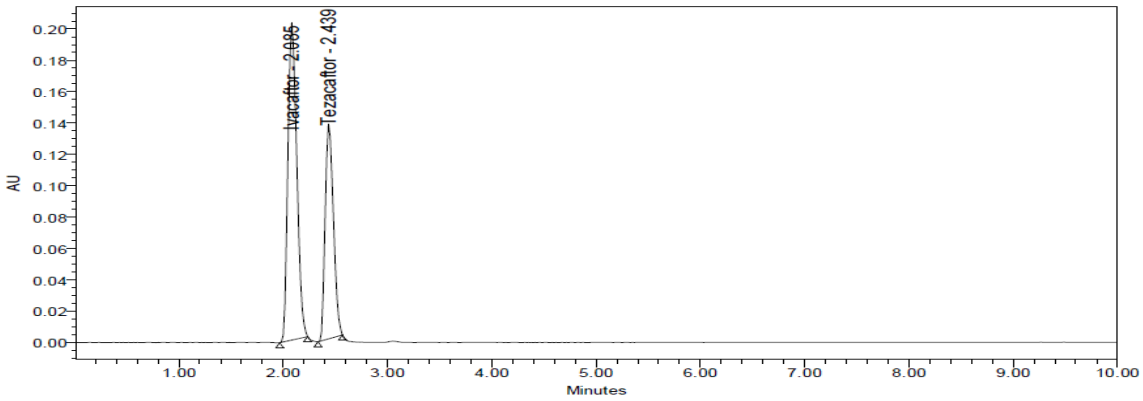


**Fig. 12: Chromatogram of Ivacaftor and Tezacaftor under neutral conditions**

**Table 1: Optimized chromatographic conditions of proposed method**

| S. No. | Parameter | Condition |
|---|---|---|
| 1 | Mobile phase | Water : acetonitrile (50:50, v/v) |
| 3 | Diluent | Water |
| 4 | Column | Kromosil $C_{18}$ column (150 mm x 4.6 mm, 5µ) |
| 5 | Column temperature | 30°C |
| 6 | Wave length | 292 nm |
| 7 | Injection volume | 10 µL |
| 8 | Flow rate | 1.0 mL/min |
| 9 | Run time | 5 min |
| 10 | Retention time | Ivacaftor -2.089 min<br>Tezacaftor-2.434 min |

**Table 2: Linearity data of Ivacaftor and Tezacaftor**

| Conc.Ivacaftor (µg/ml) | Mean area | Conc.Tezacaftor (µg/ml) | Mean area |
|---|---|---|---|
| 15 | 316571 | 10 | 206348 |
| 30 | 634445 | 20 | 392416 |
| 45 | 903945 | 30 | 579321 |
| 60 | 1178357 | 40 | 752019 |
| 75 | 1504147 | 50 | 964695 |
| 90 | 1806594 | 60 | 1173025 |

**Table 3: Precision data of Ivacaftor and Tezacaftor**

| Drug | Con (µg/ml) | Intra-day | | Inter-day | |
|---|---|---|---|---|---|
| | | Mean | %RSD | Mean | %RSD |
| Ivacaftor | 30 | 1197724 | 0.7 | 1102903 | 0.4 |
| | 60 | 1186590 | 0.6 | 1103853 | 0.3 |
| | 90 | 1187338 | 0.5 | 1092141 | 0.9 |
| Tezacaftor | 20 | 757037 | 0.2 | 708530 | 0.6 |
| | 40 | 753981 | 0.5 | 703808 | 0.8 |
| | 60 | 756768 | 0.3 | 707405 | 0.7 |

**Table 4: Accuracy data Ivacaftor and Tezacaftor**

| Drug | Con (µg/ml) | Amount recovered (µg/ml) | % Recovered | %RSD |
|---|---|---|---|---|
| Ivacaftor | 30 | 30.47 | 100.59 | 0.67 |
| | 60 | 59.54 | 101.55 | 0.24 |
| | 90 | 89.66 | 99.23 | 0.52 |
| Tezacaftor | 20 | 20.119 | 100.59 | 0.23 |
| | 40 | 39.818 | 99.55 | 0.76 |
| | 60 | 59.69 | 99.48 | 0.41 |

**Table 5: System suitability data of Ivacaftor and Tezacaftor**

| Parameter | Results | |
|---|---|---|
| | Ivacaftor | Tezacaftor |
| $R_t$ | 2.089 min | 2.434 min |
| Linearity range | 15-90 µg/ml | 10-60 µg/ml |
| C.C | 0.9994 | 0.9992 |
| N | 2977 | 4378 |
| LOD | 0.62 | 0.34 |
| LOQ | 1.88 | 1.02 |

**Table 6: Assay data of Ivacaftor and Tezacaftor**

| Drug | Labile claim | Amount found | %Recovery |
|---|---|---|---|
| Ivacaftor | 150 mg | 149.9 mg | 100.06 |
| Tezacaftor | 100 mg | 99.9 mg | 100.16 |

**Table 7: Stability-indicating data of Ivacaftor and Tezacaftor**

| Ivacaftor | | | | Tezacaftor | | |
|---|---|---|---|---|---|---|
| Condition | Std area | Sample area | % degradation | Std area | Sample area | % degradation |
| Acidic | 1197724 | 1110645 | 6.71 | 757037 | 753754 | 7.20 |
| Basic | 1197724 | 1119629 | 5.95 | 757037 | 747534 | 7.97 |
| Peroxide | 1197724 | 1155369 | 2.95 | 757037 | 760653 | 6.35 |
| Thermal | 1197724 | 1166483 | 2.02 | 757037 | 760341 | 6.39 |
| UV | 1197724 | 1173146 | 1.46 | 757037 | 799564 | 1.56 |
| Neutral | 1197724 | 1182945 | 1.46 | 757037 | 810776 | 0.18 |

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