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# Development and Validation of Tezacaftor and Ivacaftor in combined Pharmaceutical Dosage form by RP- UPLC

Gangu Sreelatha*, Jula Jeevan, Kajal Rana, V. Shruti
Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana, India – 501401

***Abstract*****—***A simple, sensitive, precise, reportable and less time-consuming method was developed for Tezacaftor and Ivacaftor using a reverse phase UPLC system. Chromatographic separation was achieved by isocratic elution on a Water Acquity UPLC CHS C-18, 50x2.1mm, 1.8µm column. The mobile phase used was 0.1% OPA (pH 2.2) and Acetonitrile in a ratio of 60:40% v/v. The flow rate was found to be 0.3mL min -1 and the detection wavelength was 292nm. The retention times of tezacaftor and ivacaftor were found to be 0.508 and 0.876 min, respectively. The % recovery was found to be between 98-102% indicating accuracy of the method. The % assay was found to be 100.10% indicating the quality and purity of the drug. The detection limits for tezacaftor and ivacaftor were found to be 0.12 and 0.27 µg mL-1, respectively. The limit of quantification (LOQ) for tezacaftor and ivacaftor was found to be 0.37 and 0.81 µg mL-1, respectively. This method has been validated according to ICH guidelines and can be used to evaluate drug purity in bulk and dosage forms.*

***Keywords*****—** *Tezacaftor, Ivacaftor, UPLC, Acetonitrile, Validation, Isocratic Elution, OPA, ICH guidelines*.

## I. INTRODUCTION

UPLC is a modern technique that provides a new direction for liquid chromatography. UPLC mainly refers to Ultra Performance Liquid Chromatography, which is developing in three areas.

- Speed
- Resolution
- Sensitivity

Ultra-performance liquid chromatography (UPLC) is used for particles with a diameter of less than 2 µm to obtain better resolution, speed and sensitivity compared to high-performance liquid chromatography (HPLC) [1-9].

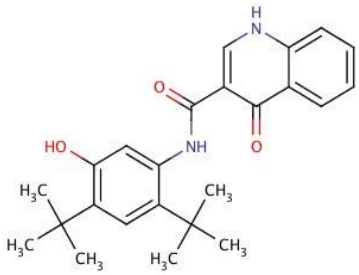


Fig. 1. Structure of Tezacaftor

Tezacaftor is a small molecule that works as a functional modulator of the fibrosis transmembrane transporter regulator (CFTR) gene. It was co-developed by Vertex Pharmaceuticals and was approved by the FDA for Ivacaftor. Chemical name 1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1- hydroxy -2-methylpropana-2-yl)-1H-indol-5-yl} cyclopropane-1-carboxamide. The molecular formula of tezacaftor is $C_{26}H_{27}F_3N_2O6$ with a molecular weight of 520.505g/mol. The transport of charged ions through the cell membrane is mediated by the cystic fibrosis transmembrane regulator (CFTR) protein. This protein acts as a channel and allows the passage of charged ions such as chlorine or sodium. (Fig 1)

Ivacaftor (also known as Kalydeco or VX-770) is a drug used to manage cystic fibrosis (CF) in patients 2 years of age and older. Chemically, N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide. It has a molecular formula of $C_{24}H_{28}N_2O_3$ with a molecular weight of 392.49 g/mol. It is sold under the brand name "SYMDEKO" [10-18]. (Fig 2)

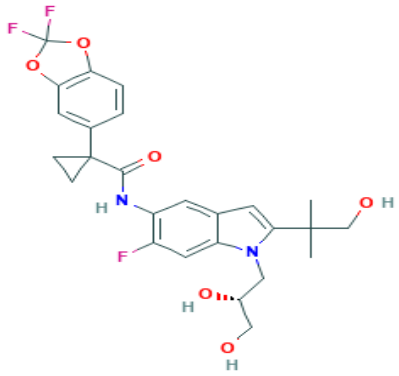


Fig. 2. Structure of Ivacaftor

A review of the literature revealed that there are many methods to determine the presence of Tezacaftor and Ivacaftor in combination with other drugs. Only a few UPLC and a few HPLC methods have been described for the simultaneous determination of tezacaftor and ivacaftor in mixed dosage forms. A simultaneous assay approach for Tezacaftor and Ivacaftor using UPLC was developed with shorter retention times than current UPLC methods [19–29].

## II. MATERIALS AND METHODS

Standard samples of tezacaftor and ivacaftor were obtained from Suven Life Sciences Ltd. Acetonitrile and



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orthophosphoric acid were of HPLC grade, sodium hydroxide and hydrogen peroxide were of GR grade (Merck Ltd. Mumbai, India). Milli-Q water was used throughout the assay.

## III. INSTRUMENTATION

The chromatographic system consisted of a Waters H-Class UPLC chromatograph (Model 2695) equipped with a CHS C18 50 X 2.1 mm 1.8 µm column, an LC-20AD pump, and an SPD-20A photo diode detector (PDA). Samples were injected into the system via a 1µL loop via a Rheodyne 7725 injector valve. The output signal was monitored and integrated by Empower-2 software. The resolution of these compounds is improved by sonication in ultrasound (PCI Analytics PCI81). All weighing in the test were performed using a Sartorius balance (model CPA225D). PVDF membrane was used for filtration and was purchased from Merck Millipore.

*Preparing the solution:*

*Preparation of the mobile phase:*

A mixture of pH 2.2 buffer and acetonitrile in a ratio of 60:40% v / v was sonicated. Transfer 1 ml of ortho phosphoric acid solution to a 1000 ml volumetric flask, add 100 ml of milli-Q water and make up to 1000 ml of milli-Q water. Water and acetonitrile were mixed in a 50:50% v/v ratio and used as a solvent to prepare the drug solution.

*Preparation of the mixed working standard solution of Tezacaftor and Ivacaftor:*

Measure and transfer the working standard of 10 mg Tezacaftor and 15 mg Ivacaftor into a clean 25 mL dry volumetric flask, add 10 mL volumetric mixture that has been sonicated for 5 minutes, and dilute the final volume (400 µg/mL Tezacaftor and 600 mL). µg/mL Ivacaftor). 5 mL of the above two stock solutions were taken in a 50 mL volumetric flask and made up to 50 mL (40 µg/mL Tezacaftor and 60 µg/mL Ivacaftor).

## IV. ASSAY

Ten tablets of SYMDEKO (Vertex Pharmaceuticals) were weighed and ground into a fine powder. Transfer the powder equal to 100 mg of tablet powder to a 250 ml volumetric flask and add 150 ml of liquid and sonicate over 30 minutes, increasing the volume. A portion of this solution was filtered through a 0.22 µm membrane filter (remove the first ml of the filter).

TABLE 1. Assay of Tezacaftor and Ivacaftor

| S. No | % Assay | |
|---|---|---|
| | Tezacaftor | Ivacaftor |
| 1 | 99.63 | 99.82 |
| 2 | 99.70 | 99.93 |
| 3 | 99.71 | 100.26 |
| 4 | 100.09 | 100.10 |
| 5 | 99.61 | 100.05 |
| 6 | 100.23 | 100.47 |
| Mean | 99.83 | 100.10 |
| SD | 0.261 | 0.23 |
| % RSD | 0.3 | 0.2 |

5 mL of the filtered solution was drawn into a 50 mL volumetric flask and diluted to 50 mL (40 µg/mL Tezacaftor and 60 µg/mL Ivacaftor). This solution (1µL) was chromatographed six times. The average peak area of the two drugs was calculated and the content in the formulation was calculated. (Table 1).

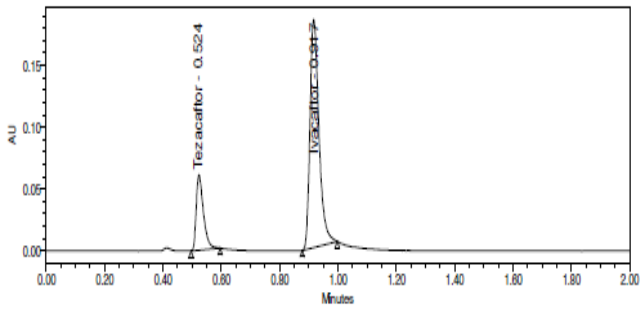


Fig. 3. Typical chromatograms of Tezacaftor and Ivacaftor in mixed sample solutions

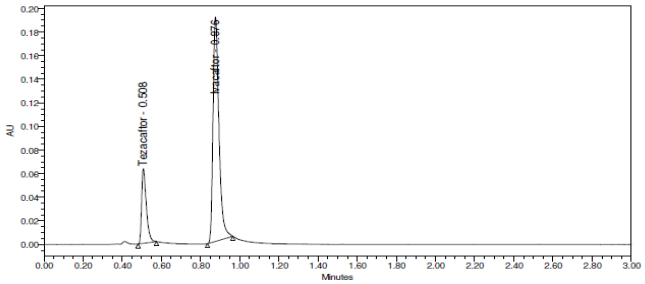


Fig. 4. Standard chromatogram of Tezacaftor and Ivacaftor in mixed standard solutions

## V. METHOD VALIDATION

The developed method was validated in accordance with ICH guidelines to adopt this procedure for system suitability, linearity, precision, accuracy and reliability, detection limit and quantification limit.

*Specificity:*

Specificity is checked by examining sample formulas for the extent to which the procedure is applicable to the analyte of interest and any interference. The specificity of the method is evaluated in terms of interference due to the presence of the receiver. The buffers used in the formulation do not interfere with the peak levels of the drug and thus the method is definitive. UPLC chromatograms recorded for the drug matrix (drug mixture) showed almost no inhibition during the retention time.

*Linearity:*

For concentration determination, a stock solution containing 500 µg/m29L Tezacaftor and 750 µg/mL Ivacaftor was prepared by dilution and further diluted to produce a solution in the concentration range of 10–60 µg/mL Tezacaftor and 15–90 µg/mL Ivacaftor. Solutions were prepared and analysed in triplicate. The experiment was repeated three times with different solution preparations and analysed by injection of 1 µL on UPLC.

*Accuracy:*



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Accuracy for Tezacaftor and Ivacaftor was performed at three different test concentration levels (i.e., 50%, 100%, and 150%) and by spraying each level with placebo powder in triplicate. Average % Recovery and % RSD values were calculated. The recovery % value was found between 98.0% and 102.0%.

*Precision:*

To determine the efficacy of the method, the system compatibility test was performed in a newly prepared standard stock solution containing 40 µg/mL Tezacaftor and 60 µg/mL Ivacaftor. 1 µL of the solution was injected into the optimized chromatography system.

Daily precisions were determined by analysing mixed solutions containing 40 µg/mL Tezacaftor and 60 µg/mL Ivacaftor. Interval accuracy was determined by different instruments on two consecutive days.

*Robustness:*

Robustness studies were conducted by using small intentional changes in the chromatographic conditions and studying the system compatibility parameters of the two drugs. The conditions chosen for the experiment were flow rate, column oven temperature, and mobile phase composition.

*Limit of detection and limit of quantification:*

The LOD and LOQ values were calculated from the mean slope and slope of the calibration curve according to ICH guidelines.

## VI. RESULTS

*System suitability:*

System suitability was evaluated by analysing mixed standard drug solutions (Tezacaftor 40 µg/mL and Ivacaftor 60 µg/mL) and calculating chromatographic parameters such as solubility, theoretical plate and tailing factor (Table 2).

TABLE 2. System suitability values for the current method

| | Parameter | Tezacaftor | Ivacaftor |
|---|---|---|---|
| 1. | Retention time (min) | 0.508 | 0.876 |
| 2. | Peak area | 104750 | 398640 |
| 3. | Resolution | - | 7.4 |
| 4. | Theoretical Plates | 2222 | 4262 |

*Linearity:*

For concentration determination, a stock solution containing 500 µg/mL Tezacaftor and 750 µg/mL Ivacaftor was prepared by dilution and further diluted to produce a solution in the concentration range of 10–60 µg/mL Tezacaftor and 15–90 µg/mL Ivacaftor (Table 3).

TABLE 3. Linearity values for the current method

| Parameter | Tezacaftor | Ivacaftor |
|---|---|---|
| Correlation Coefficient | 0.991 | 0.9991 |
| Slope | 2672 | 1782 |
| Intercept | 232 | 232 |

*Accuracy:*

To determine the accuracy of the proposed method, samples of different amounts of Tezacaftor and Ivacaftor in the linear range were taken and analysed by the proposed method (Table 4).

TABLE 4. Accuracy and recovery values for the current method

| Recovery (%level) | Tezacaftor | | Ivacaftor | |
|---|---|---|---|---|
| | % Recovery | % RSD | % Recovery | % RSD |
| 50 | 99.42 | 0.005807 | 100.55 | 0.020702 |
| 100 | 99.20 | 0.209775 | 100.24 | 0.032067 |
| 150 | 100.39 | 0.009961 | 99.58 | 0.058844 |

*Precision:*

For the suitability of the system, 6 samples of the standard solution used were injected and the maximum response of the samples was calculated (Table 5).

TABLE 5. Accuracy values for the current method

| Precision Data | Tezacaftor | | Ivacaftor | |
|---|---|---|---|---|
| | Peak Area | % RSD | Peak Area | % RSD |
| Method Precision | 107235 | 0.26 | 406882 | 0.23 |
| Intermediate Precision | 107310 | 0.88 | 407321 | 1.28 |

*Specificity:*

UPLC chromatograms recorded for the drug matrix (drug mixture) showed almost no inhibition during the retention time.

*Robustness:*

The test was performed on a mixed standard solution containing 40 µg/mL Tezacaftor and 60 µg/mL Ivacaftor. The results are not affected by small changes in these conditions (Table 6).

TABLE 6. Robustness values for the current method

| Robustness | Tezacaftor | | Ivacaftor | |
|---|---|---|---|---|
| | Rt | USP Tailing | Rt | USP Tailing |
| Flow Rate (0.2ml/min) | 0.623 | 1.6 | 1.063 | 1.6 |
| Flow Rate (0.4ml/min) | 0.446 | 1.4 | 0.751 | 1.4 |

*LOD AND LOQ:*

Results are shown in table 7.

TABLE 7. LOD and LOQ values for the current method

| S. No | Parameter | Tezacaftor | Ivacaftor |
|---|---|---|---|
| 1 | LOD | 0.12 | 0.27 |
| 2 | LOQ | 0.37 | 0.81 |

## VII. CONCLUSION

In summary, the UPLC method for Tezacaftor and Ivacaftor quantification was developed and fully validated according to ICH guidelines. This method has the advantage of less processing time than the reported method. The efficiency of using the mobile phase and the chromatogram run time of less than 1 minute make the procedure attractive. The current method shows acceptable accuracy and maximum



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sensitivity for the amount and dosage form of Tezacaftor and Ivacaftor.

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