DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TEZACAFTOR AND IVACAFTOR IN FORMULATIONS

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ABSTRACT

Objective: Aim of the present research work was to develop a sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of tezacaftor and ivacaftor in formulations.

Methods: The chromatographic separation of the mixture of tezacaftor and ivacaftor was attained in isocratic method utilizing a mobile phase of 0.1% orthophosphoric acid and acetonitrile in the proportion of 50:50%v/v utilizing a HSS C18 column which has dimensions of 100×3.0 mm, 1.5 μ particle size and the flow rate of 0.3 ml/min. The detection system was monitored at 292 nm wavelength maximum with 1.5 μl injection volume. The present method was validated as per the guidelines given by the ICH for specificity, accuracy, sensitivity, linearity and precision.

Results: The retaining time for tezacaftor and ivacaftor were achieved at 1.071 min and 0.530 min, respectively. Tezacaftor, ivacaftor and their combined drug formulation were exposed to thermal, acidic, oxidative, photolytic, and alkaline conditions. The developed method was highly sensitive, rapid, precise and accurate than the earlier reported methods. The total run time was decreased to 2.0 min; hence, the technique was more precise and economical. Stability studies directed for the suitability of the technique for degradation studies of tezacaftor and ivacaftor.

Conclusion: The projected method can be utilized for routine analysis in the quality control department in pharmaceutical trades.

Keywords: Tezacaftor, Ivacaftor, RP-UPLC, Stability studies, Validation

INTRODUCTION

Tezacaftor (TZR) and ivacaftor (IVR) drugs were combined in a single dosage form (tablet) in the brand name of symdeko, used to treat cystic fibrosis (CF) in patients more than six years old having genetically specific mutations. A wide variety of cystic fibrosis transmembrane regulator (CFTR) mutations correlate to the CF-phenotype and are accompanied with different severity stages of the disease [1, 2]. The most common mutation, affecting approximately 70% of patients with CF worldwide, is known as F508del-CFTR or delta-F508 (ΔF508), in which a deletion in the amino acid phenylalanine at 508-position resulting in impaired production of protein CFTR, thereby producing a significant decrease in the quantity of ion transporter present on cell membranes. Ivacaftor as monotherapy has failed to show a benefit for patients with delta-F508 mutations, most likely due to an insufficient amount of protein available at the cell membrane for interaction and potentiation by the drug, CFTR correctors such as tezacaftor aim to repair F508del cellular misprocessing. This is done by modulating the position of the CFTR protein on the cell surface to the correct position, allowing for adequate ion channel formation and increased in water and salt movement through the cell membrane. The concomitant use of ivacaftor is intended to maintain an open channel, increasing the transport of chloride, reducing thick mucus production [3-5].

TZR chemically designated as 1-{(2, 2-Dithieno-1, 3-benzodioxol-5-yl)-N-[(2R)-2-dihydroxypentyl]-6-fluoro-2-(2-hydroxy-1, 1-dimethyl ethyl)-1H-indol-5-yl]-cyclopropenecarboxamide with molecular weight of 520.586 g/mol. IVR chemically designated as N-(2, 4-Di-tort-butyl-5-hydroxyphenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide with molecular weight of 392.49 g/mol (fig. 1) [Rowe and Verkman, 2012; Mohan, et al, 2017]. The literature review discloses that a very few UPLC [6-8] and high performance liquid chromatographic techniques [9-13] have been reported for the estimation of TZR and IVR. Based on the reported HPLC methods, there is a need to develop a rapid, sensitive reversed-phase UPLC method for simultaneous estimation of TZR and IVR in bulk and formulations.

MATERIALS AND METHODS

Chemicals and reagents

The standard components of TZR and IVR were provided as a gift sample from MSN Laboratories, Hyderabad, India. Symdeko tablets labeled to contain TZR 100 mg and IVR 150 mg were procured from the local market. HPLC grade acetonitrile was obtained from A. B enterprises, Mumbai, India. Orthophosphoric acid was bought from Ranchem, Mumbai, India. HPLC grade water was processed by utilizing Milli-Q Millipore water purification system used during the method development.

Liquid chromatography

Chromatographic system of Waters UPLC system furnished with photodiode array detector, auto-sampler, and HSS C18 column.
which have dimensions of 100 × 2.1 mm, 1.7 μ particle size. The output signal was monitored and integrated utilizing water Empower-2.0 software. The isocratic mobile consisting of 0.1% orthophosphoric acid and acetonitrile in the proportion of 50:50%/v/v, pumped through the HSS C18 (100 × 2.1 mm, 1.7 μ) column at a fixed flow of 0.3 ml/min. The injection volume of 1.5 μl was utilized to measure the chromatograms at 292 nm as the wavelength maximum in the detection system.

Preparation of buffer
To prepare 0.1% orthophosphoric acid buffer 1 ml of orthophosphoric acid was diluted to 1000 ml with HPLC grade water.

Preparation of stock and standard solutions
Accurately Weighed and transferred 25 mg of TZR and 37.5 mg of IVR working Standards into a 25 ml clean dry volumetric flask, add 3/4 volume of diluent (Water: ACN (50:50)), sonicated for 5 min and made up to the final volume with diluent to get 1000 µg/ml of TZR and 1500 µg/ml of IVR (stock solution). 1 ml of the resulting solution was transferred into a 10 ml volumetric flask and made up to 10 ml to get 100 µg/ml of TZR and 150 µg/ml of IVR.

Preparation of sample solution
20 tablets were weighed and calculated the average weight of tablets and then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask containing 50 ml of diluent and sonicated for 25.0 min. Further, the volume made up with diluent and subjected for filtration by HPLC filters (1000 µg/ml of TZR and 1500 µg/ml of IVR). From the filtrate 1.0 ml solution was pipetted out into a 10.0 ml volumetric flask and made up to 10.0 ml with diluent to get 100 µg/ml of TZR and 150µg/ml.

Analytical method validation
The developed method for TZR and IVR was subjected for validation for the parameters like limit of detection (LOD), limit of quantification (LOQ), linearity, robustness, precision, system suitability and accuracy as per the guidelines of ICH [14-16].

RESULTS AND DISCUSSION
Optimized chromatographic conditions
After systematic trials with different mobile phase compositions and other parameters involved in the technique, HSS C18 100x 2.1 mm, 1.7μ column, isocratic mobile consisting of 0.1% orthophosphoric acid and acetonitrile in the proportion of 50:50%/v/v, column oven temperature of 30 °C, with 1.5 ml injection volume, 0.3 ml/min flow rate and detection wavelength of 292 nm were optimized. Water and acetonitrile in the ratio of 50:50 %/v was utilized as diluent.

Specificity
It is the ability of a method to unequivocally evaluate the analyte components in the presence of other components like impurities, degradants and excipients etc. expected to be present. This parameter was estimated by injecting and evaluating the blank, placebo, standard and sample solutions and chromatograms, respectively. Chromatograms of blank, placebo, and sample solution shown no peaks at the retaining time of TZR and IVR peaks. The chromatograms of TZR and IVR of standard, blank, formulation, and placebo were represented in fig. 2.

[Fig. 2: Chromatograms of A) blank, B) placebo, C) standard and D) formulation]

Linearity
Aliquots of 0.25, 0.50, 0.75, 1.0, 1.25, and 1.50 ml of standard stock solution were pipetted out from the standard stock solution of concentration 1000 µg/ml of TZR and 1500 µg/ml of IVR and made up to 10.0 ml mark with diluent. The resulting solutions were come into 25 to 150 µg/ml of TZR and 37.5 to 225 µg/ml of IVR concentration range. The resulting linearity solutions were infused into a chromatographic system and form the chromatograms linearity graph was plotted by taking the peak area on Y-axis and concentration on X-axis. The calibration graphs were shown in fig. 3 and table 1, and all findings were within limits.

| TZR | Peak area (µg/ml) | Concentration (µg/ml) | Peak area (µg/ml) |
|-----|------------------|----------------------|------------------|
| 25  | 58564            | 37.5                 | 81668           |
| 50  | 122099           | 75                   | 164210          |
| 75  | 182333           | 112.5                | 245933          |
| 100 | 245412           | 150                  | 324531          |
| 125 | 298585           | 187.5                | 403615          |
| 150 | 355250           | 225                  | 484784          |

Regression equation
y = 2384.4x+1488

Correlation coefficient (R2)
0.9999

| IVR | Peak area (µg/ml) | Concentration (µg/ml) | Peak area (µg/ml) |
|-----|------------------|----------------------|------------------|
| 37.5| 58564            | 37.5                 | 81668           |
| 75  | 122099           | 75                   | 164210          |
| 112.5| 182333           | 112.5                | 245933          |
| 150 | 245412           | 150                  | 324531          |
| 187.5| 298585           | 187.5                | 403615          |
| 225 | 355250           | 225                  | 484784          |

Regression equation
y = 2151x+1552.3

Correlation coefficient (R2)
0.9999
System suitability

Six replicates of the standard reference solution were processed and infused to perform the system suitability parameter and the resulting chromatograms peak area, retention time, resolution, plate count, and tailing were measured. The findings of the system suitability parameter were shown in table 2 and related chromatograms were given in fig. 2(C).

| S. No. | Peak name | Peak area   | Retention time | Plate count | Resolution | Tailing |
|--------|-----------|-------------|----------------|-------------|------------|---------|
| 1      | IVR       | 324491      | 0.531          | 2529        | --         | 1.41    |
| 2      | TZR       | 245595      | 1.072          | 4351        | 8.8        | 1.08    |

**LOD and LOQ**

LOD and LOQ parameters for TZR and IVR were calculated form the linear regression equation. Linearity values, graph and regression equation, were got from the linearity study and the LOD and LOQ values were represented in the table 3.

**Precision**

Analytical method precision is defined as the closeness of agreement between the replicate measurements of the analyte. It is expressed as the percentage coefficient of correlation or relative standard deviation (RSD) of the replicate measurements.

**System precision**

Working standard preparation of 1.5 µl solution was infused six times into the chromatographic system and chromatograms were obtained. %RSD of the peak area was calculated. The findings of system precision were shown in table 4.

**Method precision**

Working sample solutions of 1.5 µl were infused 6 times into the chromatographic system and chromatograms were obtained. The %RSD of the assay result of six preparations was determined. The findings achieved for assay were represented in table 5.

Table 3: Limit of detection and limit of quantification results

| Parameter | Measured concentration (µg/ml) |
|-----------|--------------------------------|
|           | TZR   | IVR   |
| LOD       | 0.41  | 0.47  |
| LOQ       | 1.23  | 1.44  |
Table 4: System precision data

| S. No. | Peak area response of drugs |
|--------|-----------------------------|
|        | TZR | IVR |
| 1      | 245595 | 325617 |
| 2      | 246876 | 329213 |
| 3      | 243399 | 325596 |
| 4      | 247294 | 324491 |
| 5      | 244267 | 324384 |
| 6      | 244682 | 326175 |
| Average| 245304 | 325913 |
| STDV   | 1522.7 | 1761.1 |
| % RSD  | 0.6   | 0.5   |

STDV: Standard deviation; RSD: Relative Standard deviation

Table 5: Method precision results

| S. No. | Peak area response of drugs |
|--------|-----------------------------|
|        | TZR | IVR |
| 1      | 249019 | 325135 |
| 2      | 243712 | 330729 |
| 3      | 245330 | 328369 |
| 4      | 243060 | 323782 |
| 5      | 243574 | 323587 |
| 6      | 244391 | 323907 |
| Average| 244848 | 325918 |
| STDV   | 2188.6 | 2959.9 |
| % RSD  | 0.9   | 0.9   |

STDV: Standard deviation; RSD: Relative Standard deviation

Table 6: Intermediate precision results

| S. No. | Peak area response of drugs |
|--------|-----------------------------|
|        | IVR | TZR |
| 1      | 279082 | 230390 |
| 2      | 278896 | 232505 |
| 3      | 286045 | 232781 |
| 4      | 282942 | 235868 |
| 5      | 284263 | 233126 |
| 6      | 286001 | 232937 |
| Average| 282872 | 232937 |
| STDV   | 3224.0 | 1750.7 |
| % RSD  | 1.1   | 0.8   |

STDV: Standard deviation; RSD: Relative Standard deviation

Table 7: Percentage recovery results

| Spiked level | IVR spiked recovery (µg/ml) | % recovery | Mean % recovery | TZR spiked recovery (µg/ml) | % recovery | Mean % recovery |
|--------------|----------------------------|------------|----------------|----------------------------|------------|----------------|
| 50%          | 50                        | 50.53892   | 101.08         | 100.21                     | 75         | 74.90          | 99.97         |
|              | 50                        | 50.4665    | 100.09         |                            | 75         | 74.55          | 99.40         |
|              | 50                        | 49.86412   | 99.73          |                            | 75         | 74.89          | 99.86         |
| 100%         | 100                       | 100.4525   | 100.45         |                            | 150        | 150.601        | 100.40        |
|              | 100                       | 101.0942   | 101.09         |                            | 150        | 150.1342       | 100.09        |
|              | 100                       | 100.3124   | 100.31         |                            | 150        | 148.3722       | 98.91         |
| 150%         | 150                       | 149.878    | 99.92          |                            | 225        | 227.7316       | 101.21        |
|              | 150                       | 149.6548   | 99.77          |                            | 225        | 225.3397       | 100.15        |
|              | 150                       | 149.2275   | 99.48          |                            | 225        | 224.5833       | 99.81         |

Intermediate precision

Working standard preparation of 1.5 µl was infused six times test preparations into the chromatographic system and chromatograms were obtained. The %RSD was evaluated for peak areas. The findings of intermediate precision study were represented in table 6.

Accuracy

A known amount of IVR and TZR at each three concentration levels of 50%, 100%, and 150% was added to a pre-analyzed sample solution and injected in triplicate at each level into the chromatographic system. The mean percentage recovery of IVR and TZR at each level was estimated. The findings were represented in tables 7.

Robustness

Working standard solution prepared as per test method was infused into the chromatographic system at variable conditions such as flow rate at±0.1 ml/min, mobile organic phase composition by±10%, and column temperature by±5 °C. The results of the robustness study parameter like peak area, retention time, plate count and tailing factor were within the limits.

Forced degradation studies

Acid degradation studies

To 1 ml of stock s solution IVR and TZR, 1 ml of 1N Hydrochloric acid were added and refluxed for 30 min at 60 °C. The resultant solution
was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 5 and table 8) [17, 18].

![Chromatogram for acid degradation study](image)

**Fig. 5: Chromatogram for acid degradation study**

| S. No. | Degradation condition | TZR | % recovery | % Degraded | IVR | % recovery | % Degraded |
|--------|-----------------------|-----|------------|------------|-----|------------|------------|
| 1      | Acid hydrolysis       | 92.16 | 7.84     |            | 91.59 | 8.41       |
| 2      | Base hydrolysis       | 93.56 | 6.44     |            | 92.82 | 7.18       |
| 3      | Peroxide              | 93.19 | 6.81     |            | 94.82 | 5.18       |
| 4      | Dry heat              | 97.98 | 2.02     |            | 96.60 | 3.40       |
| 5      | Photostability        | 98.46 | 1.54     |            | 97.88 | 2.12       |
| 6      | Water sample          | 99.51 | 0.49     |            | 99.14 | 0.86       |

**Oxidation**

To 1 ml of stock solution of VXR, SFR and VLR, 1 ml of 10% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at 60 °C. For UPLC study, the resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 6 and table 8) [18, 19].

**Alkali degradation studies**

To 1 ml of stock solution VXR, SFR and VLR, 1 ml of 1N sodium hydroxide were added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 7 and table 8).

**Dry heat degradation studies**

The standard drug solution was placed in an oven at 105 °C for 6 h to study dry heat degradation [20]. For UPLC study, the resultant solution was diluted obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 8 and table 8).

![Chromatogram for oxidation degradation study](image)

**Fig. 6: Chromatogram for oxidation degradation study**
Photo stability studies

The photochemical stability of the drug was also studied by exposing the (100 µg/ml, 400 µg/ml and 1000 µg/ml) solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt-hours/m² in photostability chamber [21]. For UPLC study, the resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 µl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 9 and table 8).

Fig. 7: Chromatogram for alkali degradation study

Fig. 8: Chromatogram for dry heat degradation study

Fig. 9: Chromatogram for photostability study
Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 °C. For UPLC study, the resultant solution was diluted to obtain 100 µg/ml of TZR and 150 µg/ml of IVR solution and 1.5 μl solution was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (fig. 10 and table 4).

Assay of marketed formulation

The marketed formulation of Symdeko (tablet) was evaluated by infusing 1.5 μl of reference and analyte solutions six times into the chromatographic system, and the resulting chromatograms of analyte were documented [22]. The quantity of analytes existed in the marketed formulation was estimated by equating the peak area of reference and analyte. The % assay of TZR and IVR was found to be 99.0–101.0%.

CONCLUSION

A sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of TZR and IVR in formulations was developed and validated as per the ICH guidelines. Retention times for TZR and IVR were achieved at 1.071 min and 0.530 min, respectively. Mean percentage recovery of TZR and IVR was found to be 100.21% and 99.97%, respectively. LOD and LOQ values obtained from regression equations of TZR and IVR and were found to be 0.41 µg/ml/1.23 µg/ml and 0.47 µg/ml/1.44 µg/ml. Regression equation of TZR and IVR were: y = 2384.4x+1480, y = 2151x+1552.3 respectively. Stability studies of these drugs proven that the percentage of degradation of analytes were found in between 0.49% to 8.41%. Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applicable in routine analysis of these drugs in quality control department of pharmaceutical trades.

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AUTHORS CONTRIBUTIONS

Lakshmi Maneka performed experiments, analysed data and co-wrote the paper. Anjana performed experiments. Saravanakumar designed and drafted the article.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of the paper.

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