APPLICATION OF UV SPECTROPHOTOMETER IN METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TEZACAFOR AND IVACAFTOR IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
Two precise, simple, accurate, reproducible, rapid and economical UV spectrophotometric methods have been developed for simultaneous estimation of tezacaftor and ivacaftor in tablet dosage form by using ethanol as solvent. Method I is based on formation and solving of simultaneous equation method, known as vierodt’s method. tezacaftor and ivacaftor show absorbance maxima at 239 nm and 351 nm respectively, so absorbance was measured at the same wavelength for the estimation of tezacaftor and ivacaftor in tablet combination. Method II is based on principle of Q-analysis, known as absorbance ratio method. Absorbance was measured at 265 nm (isobestic point) and 274 nm (λmax of ivacaftor). tezacaftor and ivacaftor obeys beer’s law in the concentration range of 5 to 30 μg/ml. Percentage estimation of tezacaftor and ivacaftor in tablet dosage form by method I is 99 and 99.85 and by method II is 99 and 99.89 respectively with standard deviation ≤2. Methods are validated according to ICH guidelines and can be applied for routine analysis of drugs in tablet dosage form.

KEYWORDS: Tezacaftor and ivacaftor.

INTRODUCTION
Ivacaftor (trade name Kalydeco) is a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (CFTR).¹¹ Ivacaftor was developed by Vertex Pharmaceuticals in conjunction with the Cystic Fibrosis Foundation
and is the first drug that treats the underlying cause rather than the symptoms of the disease.\textsuperscript{[2]}

Cystic fibrosis is caused by any one of several defects in the CFTR protein, which regulates fluid flow within cells and affects the components of sweat, digestive fluids, and mucus. Ivacaftor, a CFTR potentiator, improves the transport of chloride through the ion channel by binding to the channels directly to induce a non-conventional mode of gating which in turn increases the probability that the channel is open.\textsuperscript{[3]} Ivacaftor is approximately 99\% bound to plasma proteins.\textsuperscript{[4]}

![Chemical structure of Ivacaftor](image1)

**Tezacaftor**, also known as VX-661, is a drug approved by the FDA to treat some cases of cystic fibrosis. Tezacaftor helps move the CFTR protein to the correct position on the cell surface, and is designed to treat people with the F508del mutation.\textsuperscript{[5]}

![Chemical structure of Tezacaftor](image2)

The review of literature revealed that several methods are available for the determination of tezacaftor and ivacaftor individually. Reported methods for estimation of tezacaftor were Spectrophotometric\textsuperscript{[6]}, HPLC\textsuperscript{[7,8]}, HPTLC\textsuperscript{[9]}, and LC-MS\textsuperscript{[10]} and for ivacaftor were Spectrophotometric\textsuperscript{[11]}, HPLC\textsuperscript{[11]} and HPTLC.\textsuperscript{[12]} But there is no any analytical method yet reported for simultaneous estimation of these drugs in combination.

**UV Spectrophotometric method**

**MATERIALS AND METHODS**

UV-Visible double beam spectrophotometer, (Jasco model 2201) with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 1mm matched quartz cell was used. The commercially available tezacaftor and ivacaftor was procured from local market.
Preparation of standard stock solution and calibration curve

The standard stock solution of tezacaftor and ivacaftor were prepared by dissolving 10 mg of tezacaftor and ivacaftor in ethanol in a separate 100 ml volumetric flask and final volume was adjusted with the same solvent in 100 ml of volumetric flask to get a solution containing 100 µg/ml of tezacaftor and 100 µg/ml of ivacaftor respectively.

Working standard solutions of 10µg/ml for tezacaftor and ivacaftor were scanned in the entire UV range of 200-400 nm to determine their λmax. The λmax of tezacaftor and ivacaftor is found to be 239 nm and 351 nm respectively and isobestic point is at 265 nm from overlain spectra as shown in Fig.3. Six working standard solutions with concentration 5, 10, 15, 20, 25, 30µg/ml for tezacaftor and ivacaftor were prepared in ethanol from stock solution. The absorbance of resulting solutions were measured at their respective λmax and isobestic point and plotted a calibration curve to get the linearity and regression equation as shown in Fig. 4 and 5.

Method I (Simultaneous equation method)

Simultaneous equation method of analysis is based on the absorption of both drugs at their wavelength maximum. Two wavelengths selected for the development of the simultaneous equation are 239 nm and 351 nm. The absorptivity values were determined for tezacaftor are 0.14621 (ax1), 0.08121 (ax2) and for ivacaftor are 0.07101 (ay1), 0.1871 (ay2) at 239nm and 351 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of these drugs.

\[
\frac{C_{\text{tezacaftor}}}{ \text{Eqn.1}} = \frac{(A_2 \times a_{y2}) - (A_1 \times a_{y1})}{a_2 \times ax_1 - a_1 \times ax_2} \\
\frac{C_{\text{ivacaftor}}}{ \text{Eqn.2}} = \frac{(A_1 \times a_{y1}) - (A_2 \times a_{y2})}{a_1 \times ax_2 - a_2 \times ax_1}
\]

Where \( C_{\text{FLX}} \) and \( C_{\text{QTF}} \) are concentration of tezacaftor and ivacaftor respectively in µg/ml. \( A_1 \) and \( A_2 \) were the absorbance of the sample at 239 nm and 351 nm respectively.

Method II (Absorbance ratio method)

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isobestic point and the other being the absorption maximum of one of the two drugs. From overlain spectra (Fig.3) 265 nm (isobestic point) and 351 nm (λmax of
ivacaftor) are selected for the formation of Q absorbance equation (Eqn.3 and 4). The absorptivity values determined for tezacaftor are 0.08121 (ax1), 0.04958 (ax2) and for ivacaftor are 0.17710 (ay1), 0.17794 (ay2) at 351 nm and 265 nm respectively. These values are means of six estimations. The absorbance and absorptivities at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of these drugs.

\[
C_{\text{tezacaftor}} = \frac{Q_M - Q_X}{Q_Y - Q_X} \frac{A_1}{ax_1} \quad \text{Eqn.3}
\]

\[
C_{\text{ivacaftor}} = \frac{Q_M - Q_Y}{Q_X - Q_Y} \frac{A_1}{ay_1} \quad \text{Eqn.4}
\]

Q_M, Q_X and Q_Y were obtained from Eqn.no.5, 6, 7 respectively.

\[
Q_M = \frac{A_2}{A_1} \quad \text{Eqn.5}
\]

\[
Q_X = \frac{ax_2}{ax_1} \quad \text{Eqn.6}
\]

\[
Q_Y = \frac{ay_2}{ay_1} \quad \text{Eqn.7}
\]

Where \(C_{\text{FLX}}\) and \(C_{\text{QTF}}\) are concentration of tezacaftor and ivacaftor respectively in \(\mu\text{g/ml}\). \(A_1\) and \(A_2\) were the absorbance of the sample at 265 nm and 351 nm respectively.

**Validation of developed methods**

**Linearity**

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed method. For method I and II, the Beer-Lambert’s concentration range was found to be 5-30 \(\mu\text{g/ml}\) for both tezacaftor and ivacaftor. The linearity data of both methods are presented in Table.1.

**Accuracy**

To check the accuracy of the proposed methods, recovery studies were carried out at 80, 100 and 120\% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are shown in Table.2.

**Repeatability**

To check the degree of repeatability of these methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with synthetic mixture. The standard
deviation and coefficient of variation were calculated. The results of statistical evaluation are given in Table.2.

**Intermediate Precision (Interday and Intraday precision)**

The Interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table.3.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ of tezacaftor and ivacaftor by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are quoted in Table.3.

![Fig. 3: Overlain spectra of tezacaftor and ivacaftor.](image1)

![Fig. 4: Calibration curve and regression equation of Tezacaftor in ethanol.](image2)
Fig. 5: Calibration curve and regression equation of Ivacaftor in ethanol.

Table 1: Optical Characteristics for tezacaftor and ivacaftor.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tezacaftor</th>
<th>Ivacaftor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working wavelength</td>
<td>283nm</td>
<td>285nm</td>
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<tr>
<td>Beer’s law limit(µg/ml)</td>
<td>5-30</td>
<td>5-30</td>
</tr>
<tr>
<td>Absorptivity*</td>
<td>0.1462</td>
<td>0.0495</td>
</tr>
<tr>
<td>Correlation coefficient*</td>
<td>0.999</td>
<td>0.997</td>
</tr>
<tr>
<td>Intercept*</td>
<td>0.008</td>
<td>0.026</td>
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<tr>
<td>Slope*</td>
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<td>0.047</td>
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</table>

*Average of six estimations.

Table 2: Recovery studies.

<table>
<thead>
<tr>
<th>Method</th>
<th>Level of recovery (%)</th>
<th>% Recovery ±S.D.#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tezacaftor</td>
</tr>
<tr>
<td>I</td>
<td>80</td>
<td>98.5±0.45</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.42±0.60</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>99.60±0.97</td>
</tr>
<tr>
<td>II</td>
<td>80</td>
<td>98.56±0.54</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.45±0.65</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>100.56±0.78</td>
</tr>
</tbody>
</table>

#mean of three determinations, SD: Standard Deviation

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REFERENCE


