DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF LEVOCETIRIZINE DIHYDROCHLORIDE AND MONTELUKAST SODIUM IN TABLET DOSAGE FORM

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ABSTRACT

Four simple and accurate spectrophotometric methods have been described for the simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in tablet dosage form. The first method is a bivariate calibration algorithm involving the use of two selected wavelengths 220 nm and 230 nm for the determination of studied drugs. The second method is a dual wavelength which is based on determination of levocetirizine dihydrochloride at the absorbance difference between 208 nm and 214.4 nm in which montelukast sodium absorbance difference was zero for any concentration, also montelukast sodium can be determined at absorbance difference between 355 and 390 nm in which levocetirizine dihydrochloride has absorbance zero for any concentration also. The third method is a derivative spectrophotometry, in which the two drugs were quantified using second derivative responses at 244 nm for levocetirizine dihydrochloride and at 293.2 or 335.6 nm for montelukast sodium. The fourth method is ratio difference which depends on measuring the peak amplitudes for levocetirizine dihydrochloride at 216 and 232 nm using 4 μg mL⁻¹ montelukast sodium as a divisor, while montelukast sodium can be measured at 296.4 and 344.2 nm using 4 μg mL⁻¹ of levocetirizine dihydrochloride as divisor. Beer’s law was obeyed in the concentration range 4–28 μg mL⁻¹ for both drugs in all methods. The developed methods were used to determine the studied drugs in bulk powder, laboratory prepared mixtures and pharmaceutical dosage form with good accuracy and precision. All methods were validated.
according to ICH guidelines and the results obtained were statistically compared to those obtained from a reference method and were found to be in good agreement.

KEYWORDS: Levocetrizine dihydrochloride, Montelukast sodium, Bivariate, Dual wavelength, Second derivative, Ratio difference.

1. INTRODUCTION

Levocetirizine (LCZ), is a third-generation non-sedative antihistamine used for the treatment of allergic rhinitis and chronic idiopathic urticaria \[^{1}\], chemically described as 2-[2-[4-[(R)-(4-chlorophenyl) - phenyl-methyl] piperazin-1-yl] ethoxy] acetic acid \[^{2}\]. It is an active Renantiomer of cetirizine, orally active, potent, selective and long acting H1-histaminereceptorantagonist with no anticholinergic activity \[^{2}\]. Montelukast (MLK) is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene receptor in the lungs and bronchial tubes. It is used for the treatment of asthma and to relieve symptoms of seasonal allergies \[^{3}\]. MLK described chemically as 2-[1-[[1R]-1- [3- [2- (7-chloroquinolin-2-yl) ethenyl]phenyl]-3- [hydroxypropan-2yl] phenyl] propyl] sulfanyl methyl] cyclopropyl]acetic acid; Figure (1).

Several analytical methods were reported for the determination of LCZ either alone or in combination with other drugs including UV spectrophotometric\[^{4-7}\] and HPLC \[^{8-11}\]. Whereas, MLK was determined alone or in combined dosage form by UV spectrophotometric \[^{12}\], capillary electrophoresis \[^{13}\], Voltammetric\[^{14}\] and HPLC methods \[^{15-18}\]. Literature survey for simultaneous determination of LCZ and MLK in their binary mixture revealed UV spectrophotometric\[^{19-21}\] HPLC\[^{22-24}\] and HPTLC\[^{21,24}\] methods. The present work describes newly developed and validated spectrophotometric methods for simultaneous estimation of LCZ and MLK in bulk powder and in tablets.

![Figure 1: Chemical structures of LCZ and MLK](image-url)
2. MATERIALS AND METHODS

2.1. Instrumentation
Shimadzu, UV-Vis 1601 PC spectrophotometer (Tokyo, Japan), connected with UV probe program with two matched 1 cm path-length quartz cell.

2.2. Samples
2.2.1. Pure samples
LCZ; batch no. LCZ-1304009 and MLK; batch no. MK-0180513 pure samples were kindly supplied by EGY Pharm. Its purity was found to 99.78 or 99.30%, respectively as stated by the supplier.

2.2.2. Market sample
Montair-LC® tablets; batch no. D3184-8, labeled to contain 5 mg of LCZ and 10 mg of MLK, cipla, India.

2.3. Chemicals and reagents
All reagents used were of analytical grade and solvents were of spectroscopic grade. Methanol (Sigma- Aldrich, Germany, Riedel-deHaen, Sleeze- Germany and Biotec for laboratory chemicals).

2.4. Standard solutions
- Standard stock solutions of the drugs (0.4 mg mL⁻¹) were prepared by dissolving 10 mg of LCZ or MLK in 25 mL methanol.

- Working standard solutions of LCZ and MLK were prepared by further dilution of standard stock solution with methanol as appropriate to obtain the working concentration range.

2.5. Procedures
2.5.1. Bivariate method
2.5.1. 1. Construction of calibration curve
Aliquots of standard drug solutions (0.4 mg mL⁻¹) equivalent to (4-28 μg mL⁻¹) of LCZ or MLK were transferred separately into two sets of 10 mL volumetric flasks, completed to the mark with methanol. The zero order spectra of LCZ and MLK solutions were recorded in the range of 200-400 nm. Calibration curves at different wavelengths 220-240 nm at 2 nm interval were constructed, the corresponding regression equations and the sensitivity matrices K were calculated; Table (1).
From which the optimum pair of wavelengths to carry out the determination and the four regression equations used in the bivariate algorithm were investigated; Table (2).

2.5.1.2. Assay of laboratory prepared mixtures
Different aliquots volumes of LCZ and MLK (0.4 mg mL$^{-1}$) were transferred into a series of 10 mL volumetric flasks and diluted to the volume with methanol. The spectra of the prepared solutions were recorded at 220 and 230 nm. The concentrations of the two drugs were calculated using the following equations\cite{25}:

$$C_A = \frac{(A_{AB1} - e_{AB1} - m_{B1}C_B)}{m_{A1}}$$

$$C_B = \frac{[m_{A2} (A_{AB1} - e_{AB1}) + m_{A1} (e_{AB2} - A_{AB2})]}{m_{A2}m_{B1} - m_{A1}m_{B2}}$$

Where $C_A$, $C_B$ are the concentration of component A (LCZ), component B (MLK); $m_{A1}$, $m_{A2}$ are the slope values of LCZ at $\lambda_1$, $\lambda_2$; $m_{B1}$, $m_{B2}$ are the slope values of MLK at $\lambda_1$, $\lambda_2$; $A_{AB1}$, $A_{AB2}$ are the absorbance of the binary mixture at $\lambda_1$, $\lambda_2$; $e_{AB1}$, $e_{AB2}$ are the sum of the intercepts of LCZ and MLK at $\lambda_1$ and $\lambda_2$, respectively.

2.5.2. Dual wavelength spectrophotometric method

2.5.2.1. Construction of calibration curve
Into two separate sets of 10 mL volumetric flasks, aliquots of LCZ or MLK standard solutions (0.4 mg mL$^{-1}$) containing 4-28 μg mL$^{-1}$ of either LCZ or MLK were pipetted and diluted to the volume with methanol. Absorbance difference of each solution was measured at 208 and 214.4 nm or at 355 and 390 nm for LCZ or MLK, respectively. Calibration curves were constructed by plotting the absorbance difference versus concentration in μg mL$^{-1}$ and the regression equations were computed.

2.5.2.2. Assay of laboratory prepared mixtures
Different aliquot volumes of LCZ and MLK (0.4 mg mL$^{-1}$) were introduced into a series of 10 mL volumetric flasks and adjusted to the volume with methanol. Absorbance difference between 208 and 214.4 nm or 355 and 390 nm were measured and then the concentrations of LCZ or MLK, respectively in the prepared mixtures were calculated from the corresponding regression equation.
2.5.3. Second derivative spectrophotometric method

2.5.3.1. Construction of calibration curve
Appropriate aliquots of LCZ or MLK standard solutions (0.4 mg mL\(^{-1}\)) containing 4-28 μg mL\(^{-1}\) of either LCZ or MLK were accurately transferred into different 10 mL volumetric flasks and diluted to the volume with methanol. Second derivative spectra of the drugs were recorded at 244 nm or at 293.2 and 335.6 nm for LCZ or MLK, respectively with Δλ = 4 nm and a scaling factor = 20 against methanol. Calibration graphs were constructed by plotting peak amplitude versus drug concentration.

2.5.3.2. Assay of laboratory prepared mixtures
Aliquot portions of LCZ and MLK (0.4 μg mL\(^{-1}\)) are pipetted into a series of 10 mL volumetric flasks and diluted to the volume with methanol. The obtained solutions were analysed by the proposed method by scanning the calibration curves at 244 nm or at 293.2 and 335.6 nm for both LCZ and MLK, respectively. The concentration of each drug in the prepared mixtures was calculated from the regression parameters.

2.5.4. Ratio difference method

2.5.4.1. Construction of calibration curve
Aliquots of standard drug solutions (0.4 mg mL\(^{-1}\)) equivalent to 4-28 μg mL\(^{-1}\) of LCZ or MLK were introduced into two separate series of 10 ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions were scanned from 200-400 nm and stored in the computer. The stored spectra of LCZ were divided by the spectrum of 4 μg mL\(^{-1}\) MLK, while MLK spectra were divided by the spectrum of 4 μg mL\(^{-1}\) LCZ. Calibration curves for LCZ and MLK were constructed by plotting the difference between the amplitudes of ratio spectra at 216 and 232 nm or at 296.4 and 344.2 nm versus the corresponding concentrations for both LCZ and MLK, respectively.

2.5.4.2. Assay of laboratory prepared mixtures
Accurately measured aliquot volumes of LCZ and MLK (0.4 mg mL\(^{-1}\)) were introduced into a series of 10 ml volumetric flasks and diluted to the volume with methanol. The differences between the amplitudes of ratio spectra were recorded at 216 and 232 nm or at 296.4 and 344.2 nm for LCZ and MLK, respectively. The concentration of both LCZ and MLK in the prepared mixtures was calculated from the corresponding regression parameters.
2.5.4. Application to pharmaceutical preparation

Five Montair-LC® tablets each labeled to contain 5 mg LCZ and 10 mg of MLK were weighed, powdered and mixed well. An accurately weighed quantity of the powder equivalent to one tablet was introduced into a 25 mL volumetric flask. Volume was adjusted up to the mark with the methanol. The flask was sonicated for 15 minutes and then filtered.

The clear filtrate claimed to contain 0.2 mg mL\(^{-1}\) of LCZ and 0.4 mg mL\(^{-1}\) of MLK to be analyzed by the proposed methods. The drug concentrations were calculated from the appropriate regression parameters.

3. RESULTS AND DISCUSSION

Four different analytical procedures were developed; bivariate, dual wavelength, second derivative and ratio difference methods aiming for the simultaneous determination of LCZ and MLK.

3.1. Bivariate method

Bivariate calibration spectrophotometric method is a direct method which has been proposed for the resolution of binary mixtures. This method is based on the simple mathematic algorithm, in which the data were used from four regression equations, two calibrations for each component at two wavelengths selected using Kaiser method \(^{[25,26]}\). The principle of bivariate calibration is in the measurements of binary mixture (A and B) at two carefully selected wavelengths (\(\lambda_1\) and \(\lambda_2\)), to obtain two equations\(^{[25]}\):

\[
\begin{align*}
A_{AB1} &= m_{A1}C_A + m_{B1}C_B + e_{AB1} \\
A_{AB2} &= m_{A2}C_A + m_{B2}C_B + e_{AB2}
\end{align*}
\]

The resolution of such equations, allows the evaluation of \(C_A\) and \(C_B\) values according to the previously mentioned equations. In order to apply the bivariate method in the resolution of binary mixture of LCZ and MLK, the absorbance of the two components at several different selected wavelengths was recorded in the region of overlapping; from 220 to 230 nm at 2 nm interval. The calibration curve equations and their respective linear regression coefficients were obtained to ensure a linear relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths showed a satisfactory linear regression coefficient (\(r^2 > 0.9978\)).

The slope values of the linear regression equations for both LCZ and MLK at the selected wavelengths were used according to Kaiser method to calculate the sensitivity matrices K to
find out the optimum pair of wavelength at which the binary mixtures were recorded; Table (1).

![K matrix image]

Table (1): Values of the sensitivity matrix determinates calculated according to Kaiser’s method (k x 10⁻⁶) for the mixture of LCZ and MLK by the proposed bivariate method.

<table>
<thead>
<tr>
<th>λ/λ</th>
<th>220</th>
<th>222</th>
<th>224</th>
<th>226</th>
<th>228</th>
<th>230</th>
<th>232</th>
<th>234</th>
<th>236</th>
<th>238</th>
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<tr>
<td>222</td>
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<td></td>
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<tr>
<td>224</td>
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<td>-219.52</td>
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<td></td>
</tr>
<tr>
<td>226</td>
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<td>-448.24</td>
<td>-231.74</td>
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<td></td>
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<tr>
<td>228</td>
<td>-818.86</td>
<td>-682.2</td>
<td>-479.03</td>
<td>-257.96</td>
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<td></td>
</tr>
<tr>
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<td>-623.18</td>
<td>-413.84</td>
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<td></td>
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<td>-819.04</td>
<td>-638.36</td>
<td>-438.84</td>
<td>-196.54</td>
<td>-40.12</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>234</td>
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<td>-657.04</td>
<td>-485.22</td>
<td>-297.16</td>
<td>-74.14</td>
<td>67.4</td>
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<td></td>
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<tr>
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<td>-333.28</td>
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<td>07.24</td>
<td>202.82</td>
<td>320.84</td>
<td>339.52</td>
<td>231.56</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>238</td>
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<td>-359.2</td>
<td>115.02</td>
<td>272.78</td>
<td>435.83</td>
<td>528.14</td>
<td>533.6</td>
<td>423.66</td>
<td>202.24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>133.38</td>
<td>231.08</td>
<td>369.45</td>
<td>510.44</td>
<td>643.88</td>
<td>712.88</td>
<td>706.46</td>
<td>595.02</td>
<td>383.26</td>
<td>181.5</td>
<td>0</td>
</tr>
</tbody>
</table>

For the bivariate determination of LCZ and MLK at 220 and 230 nm were found to give the maximum value of K and thus can be used for the analysis; Figure (2). The linear regression formulas used for the bivariate algorithm were presented in table (2).

Table(2): Linear regression calibration formula used for the bivariate algorithm.

<table>
<thead>
<tr>
<th>Component</th>
<th>Calibration equations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at λ₂₂₀</td>
</tr>
<tr>
<td>LCZ</td>
<td>A = 0.0226 C + 0.00717</td>
</tr>
<tr>
<td></td>
<td>r² = 0.9970</td>
</tr>
<tr>
<td>MLK</td>
<td>A = 0.05527 C + 0.0557</td>
</tr>
<tr>
<td></td>
<td>r² = 0.9970</td>
</tr>
</tbody>
</table>
Figure 2: Absorption spectra of 8 μg mL⁻¹ LCZ (——), 4 μg mL⁻¹ MLK (……) and (4:8 μg mL⁻¹) mixture of both drugs (--- . --) in methanol.

3.2. Dual wavelength spectrophotometric method
The proposed dual wavelength spectrophotometric method was developed for the simultaneous determination of LCZ and MLK. The principle for dual wavelength method is the absorbance difference between two points on the overlain spectra is directly proportional to the concentration of the component of interest. This method offer an efficient method for analysis of binary mixtures where dual analytical wavelengths were selected in a way to make the absorbance difference zero for one drug in order to analyze the other drug [27,28].

The difference in absorbance at 208 and 214.4 nm was zero for MLK so they were selected for determination of LCZ, whereas the difference in absorbance at 355 and 390 nm was zero for LCZ and hence they were used to analyze MLK; Figure (2).

3.3. Second derivative spectrophotometric method
The third method eliminating the interference in spectra is the second derivative method. The zero-order spectra of the two cited drugs showed sever overlapping; Figure (2). However, conversion of zero-order spectra to second derivative spectra permitted the determination of LCZ at 244 nm and MLK at 293.2 or 335.6 nm; Figure (3).
3.4. Ratio difference method

Ratio difference is a new and simple method for the simultaneous determination of components with overlapping spectra in binary mixtures, having the advantages of minimal data processing and wider range of application. The method comprises two critical steps; the first is the choice of the divisor and the selected divisor should compromise between minimal noise and maximum sensitivity, the second critical step is the choice of the wavelengths at which measurements are recorded [29].

The difference between amplitudes in the ratio spectra at 216 and 232 nm or 296.4 and 344.2 nm were selected for determination of LCZ or MLK, respectively; Figure (4).

Figure 3: Second derivative spectra of 4 μg mL⁻¹ LCZ (—), 4 μg mL⁻¹ MLK (……) in methanol.

Figure 4: Ratio spectra of 28 μg mL⁻¹ LCZ using 4 μg mL⁻¹ MLK as divisor (—) and 4 μg mL⁻¹ MLK using 4 μg mL⁻¹ LCZ as divisor (……).
3.5. Methods Validation

3.5.1. Linearity

The linearity of the proposed methods was evaluated through the analysis of serial concentrations of each drug. The produced response was plotted as a function of the corresponding concentration in which the linearity range is 4-28 μg mL\(^{-1}\) for the two cited drugs in all studied methods; Table (3).

3.5.2. LOD and LOQ

The experimental limits of detection (LOD) and limits of quantification (LOQ) were calculated according to the ICH guidelines\(^{[30]}\) using the standard deviation of multiple blank samples and the slope of the calibration curve; Table (3).

3.5.3. Accuracy and Precision

The accuracy and precision of the proposed methods were assessed using three different concentrations of pure samples of the drug covering the linearity range, each in triplicate, within one day for intraday analysis and different three days for interday analysis. % RSD values for the four methods were found to be less than 2% indicating good precision of the developed methods; Table (3).
Table (3): Spectral data of calibration curves for the determination of LCZ and MLK by the proposed methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bivariate Method</th>
<th>Dual wavelength Method</th>
<th>Second derivative Spectrophotometric Method</th>
<th>Ratio difference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCZ</td>
<td>MLK</td>
<td>LCZ</td>
<td>MLK</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>220, 230</td>
<td>208, 214.4</td>
<td>244</td>
<td>355, 390</td>
</tr>
<tr>
<td>Linearity range (( \mu g \text{ mL}^{-1} ))</td>
<td>4-28</td>
<td>4-28</td>
<td>4-28</td>
<td>4-28</td>
</tr>
<tr>
<td>Regression parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Slope</td>
<td>0.0345</td>
<td>0.0462</td>
<td>0.0256</td>
<td>0.04052</td>
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<tr>
<td>Intercept</td>
<td>0.0364</td>
<td>-0.0284</td>
<td>0.02139</td>
<td>-0.0440</td>
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<td>Correlation coefficient ((r^2))</td>
<td>0.9991</td>
<td>0.9992</td>
<td>0.9990</td>
<td>0.9989</td>
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<tr>
<td>LOD</td>
<td>0.261</td>
<td>0.079</td>
<td>0.374</td>
<td>0.273</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.893</td>
<td>0.264</td>
<td>1.249</td>
<td>0.910</td>
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<tr>
<td>Accuracy (R%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>98.94</td>
<td>99.67</td>
<td>98.77</td>
<td>100.24</td>
</tr>
<tr>
<td>Interday</td>
<td>99.02</td>
<td>100.17</td>
<td>99.60</td>
<td>100.15</td>
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<tr>
<td>Precision (RSD %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>0.0640</td>
<td>0.0133</td>
<td>0.0156</td>
<td>0.0089</td>
</tr>
<tr>
<td>Interday</td>
<td>0.0153</td>
<td>0.0970</td>
<td>0.0124</td>
<td>0.0091</td>
</tr>
</tbody>
</table>
3.5.4. Selectivity

Methods selectivity was assured by analyzing laboratory prepared mixtures of the two studied drugs at different ratios within the linearity range. Good recoveries of both drugs indicate high selectivity of the proposed methods for simultaneous determination of LCZ and MLK in binary mixtures; Table (4).

Table (4): Determination of LCZ and MLK in laboratory prepared mixtures by the proposed methods.

<table>
<thead>
<tr>
<th>LCZ/MLK Ratio</th>
<th>Bivariate Method</th>
<th>Dual wavelength Method</th>
<th>Derivative spectrophotometric method</th>
<th>Ratio difference Method</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Recovery %</td>
<td>Recovery %</td>
<td>Recovery %</td>
<td>Recovery %</td>
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<tr>
<td></td>
<td>LCZ</td>
<td>MLK</td>
<td>LCZ</td>
<td>MLK</td>
</tr>
<tr>
<td>1 : 2</td>
<td>97.25</td>
<td>99.87</td>
<td>100.00</td>
<td>99.00</td>
</tr>
<tr>
<td>1 : 1</td>
<td>100.00</td>
<td>102.00</td>
<td>99.75</td>
<td>98.50</td>
</tr>
<tr>
<td>2 : 1</td>
<td>101.00</td>
<td>99.87</td>
<td>97.60</td>
<td>98.37</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>99.41 ± 1.94</td>
<td>100.58 ± 1.22</td>
<td>99.11 ± 1.31</td>
<td>98.62 ± 0.33</td>
</tr>
</tbody>
</table>

3.5.5. Robustness

The robustness of the proposed methods was checked by studying the effect of different sources of methanol. It was found that, using methanol of Sigma- Aldrich, Riedel-deHaen and Biotec for laboratory chemicals gave RSD% did not exceed 0.029%.

3.5.6. Stability of standard solutions

The stability of LCZ and MLK solutions was evaluated by analyzing of two different solutions; one of them was kept at room temperature while the other was kept in refrigerator against freshly prepared standards. The results showed that both drugs were stable for one week either kept at room temperature or in refrigerator.

3.4. Application to pharmaceutical preparation

The proposed methods were successfully applied for the simultaneous determination of both LCZ and MLK in pharmaceutical preparation without interference of the additives present.

Satisfactory results were obtained for each drug in good agreement with the label claim (mean recovery ranging from 97.87 to 101.89%). The recovery of the proposed procedures was determined by applying the standard addition technique; Table (5). Statistical analysis of the results obtained by the proposed methods compared with a reported method^{19} revealed...
no significant difference between the proposed and reported methods confirming accuracy and precision at 95% confidence limit$^{[31]}$, Table (5).
Table (5): Results obtained by the proposed methods compared with reported method\(^{[19]}\) for determination of LCZ and MLK in pharmaceutical dosage form.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bivariate Method</th>
<th>Dual wavelength Method</th>
<th>Derivative Spectrophotomeric Method</th>
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<td>LCZ</td>
<td>MLK</td>
<td>LCZ</td>
<td>MLK</td>
<td>LCZ</td>
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<tr>
<td>Linearity range (µg mL(^{-1}))</td>
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<td>4 -28</td>
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<tr>
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<td>4</td>
<td>4</td>
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<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Mean %</td>
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<td>97.87</td>
<td>99.85</td>
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<td>99.24</td>
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<td>SD</td>
<td>0.96</td>
<td>0.93</td>
<td>1.53</td>
<td>1.98</td>
<td>1.03</td>
</tr>
<tr>
<td>Variance</td>
<td>0.92</td>
<td>0.86</td>
<td>2.34</td>
<td>3.92</td>
<td>1.06</td>
</tr>
<tr>
<td>t</td>
<td>0.31 (2.45)</td>
<td>0.41 (2.45)</td>
<td>0.46 (2.45)</td>
<td>0.11 (2.45)</td>
<td>0.72 (2.57)</td>
</tr>
<tr>
<td>F</td>
<td>3.63 (9.28)</td>
<td>2.83 (9.28)</td>
<td>1.43 (9.28)</td>
<td>1.61 (9.28)</td>
<td>3.15 (19.2)</td>
</tr>
<tr>
<td>Standard added mean + SD %</td>
<td>98.94 ±1.17</td>
<td>98.82 ±1.01</td>
<td>98.82 ±1.01</td>
<td>100.16 ±1.12</td>
<td>99.20 ±1.48</td>
</tr>
</tbody>
</table>

- Figures in parenthesis are the theoretical t and F values at p = 0.05.
- Reported method\(^{[19]}\) is a first derivative of the ratio spectra depends on measurement of the amplitudes at 238.4 nm for LCZ using 8 µg mL\(^{-1}\) of MLK as a divisor and at 250.4 nm for MLK using 4 µg mL\(^{-1}\) of LCZ as a divisor, respectively.
CONCLUSION

The proposed study describes four different methods for estimation of levocetirizine dihydrochloride and montelukast sodium in bulk or in their combination; bivariate, dual wavelength, second derivative and ratio difference spectrophotometric methods. All methods were validated and found to be simple, sensitive, accurate, precise and devoid from any potential interference. Therefore, they can be conveniently adopted for estimation and routine quality control analysis of both drugs.

REFERENCES


30. ICH, Q2B In proceedings of The International Conference on Harmonization, Geneva 1993.