Stability-Indicating Determination of Rebamipide in the Presence of its Acid Degradation Products

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Four sensitive and precise stability-indicating methods for the determination of rebamipide (REB) in the presence of its acid-degradation products and in a pharmaceutical formulation were developed and validated. Method A used the first derivative of the ratio spectra (1DD) spectrophotometric method by measuring the peak amplitude at 249.4 nm (maximum) and at 259 nm (minimum), and at the total peak amplitude (from 249.4 to 259 nm, 1DD 249.4 + 259 nm) in the range of 2–14 µg/mL. This method yielded mean recoveries of 99.87 ± 0.83, 100.04 ± 0.75, and 100.28 ± 1.11%, respectively.

Method B is a dual wavelength method, which allows the determination of REB in presence of its acid-degradation products by measuring the absorbance difference between 254 and 269 nm within a linearity range of 5–65 µg/mL; it showed a mean recovery of 99.84 ± 1.06. Method C is a TLC-densitometric procedure in which REB was separated from its degradation products using a developing solution of methanol–chloroform–ammonia (8.5 ± 1.5 + 0.5, v/v/v). The quantitative evaluation of REB at 329 nm was linear over the range of 0.50–4.5 µg/mL, with a mean recovery of 99.49 ± 0.99% even in the presence of up to 90% degradation products. Method D is an RP-HPLC procedure. It provided the complete separation of REB from its degradation products on an Xterra® C18 column using phosphate buffer (pH 6, 0.01 M)–methanol (1 + 1, v/v) as the mobile phase (UV detection at 254 nm). Recovery was 99.28 ± 0.78% within the range of 10–190 µg/mL.

The selectivity of the proposed methods was checked using laboratory-prepared mixtures. The proposed methods have been successfully applied to the analysis of REB in pharmaceutical dosage forms without interference from other dosage form excipients.

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from E. Merck, and o-phosphoric acid (85%) from Analar BDH-Laboratory Suppliers (Poole, UK). Deionized, bi-distilled water was from an Aquatron Automatic Water Still A4000 (Bibby Sterillin Ltd, Staffordshire, UK).

The REB standard was kindly supplied by Egypt Otsuka Pharmaceutical Co., S.A.E. (Cairo, Egypt). Its purity 99.78% according to the reported HPLC method (6). All chemicals used throughout this work were of analytical grade, and solvents were of spectroscopic grade.

**Pharmaceutical Formulation**

Mucosta® tablets labeled to contain 100 mg of REB were manufactured by Egypt Otsuka Pharmaceutical Co., S.A.E., under license from Otsuka Pharmaceutical Co., Ltd (Tokyo, Japan). Batches No. 9692 and 7451 were purchased from the Egyptian markets.

**Degraded Sample**

Accelerated acid-degradation was performed by dissolving 25 mg of pure REB powder in 5 mL methanol; then 25 mL of 5 N hydrochloric acid was added and the solution was refluxed for 5 h. Complete degradation was followed by TLC using a methanol–chloroform–ammonia developing solution (8.5 +1.5 + 0.5, v/v/v). Degradant 1 was extracted by chloroform. Degradant 2 was precipitated at pH 6.4 ± 0.2 by 5 N sodium hydroxide, washed, and subsequently recrystallized from methanol. The degradation products were elucidated by IR and MS.

**Standard Solutions**

(a) Stock standard solutions of REB were 0.1 mg/mL in methanol (for 1DD, dual wavelength, and TLC densitometric methods) and 0.2 mg/mL in the mobile phase (for the HPLC method).

(b) Stock standard solutions of the acid degradation products derived from the complete degradation of the 0.1 mg/mL standard REB solution in methanol 0.1 mg/mL (for 1DD, dual wavelength, and TLC densitometric methods) and the 0.5 mg/mL standard solution of REB in the mobile phase (for the HPLC method).

All stock standard solutions were freshly prepared on the day of analysis and stored in the refrigerator to be used within 24 h.

**Spectrophotometric Methods: Calibration Curve for 1DD Method**

Aliquots of the REB stock solution (0.1 mg/mL) equivalent to 20–140 µg/mL were accurately transferred into a series...
of 10 mL volumetric flasks, then diluted to volume with methanol. 1DD curves were recorded at Δλ = 4 nm and a scaling factor = 15. The absorption spectra of this solution was divided by the absorption spectrum from 20 µg/mL of the acid degradation products (as a divisor). The obtained ratio spectra was then differentiated with respect to wavelength. The peak amplitudes at 249.4 and 259 nm, and 1DD (249.4 + 259 nm) were recorded. Calibration graphs were constructed relating the peak amplitudes of (1DD) to the corresponding concentrations. The regression equations were then computed for the studied drug at the specified wavelengths and used for the determination of unknown samples containing REB.

Dual Wavelength Method: Calibration Curve

Different aliquots equivalent to 50–650 µg of REB stock solution (0.1 mg/mL) were accurately transferred into a series of 10 mL volumetric flasks, then diluted to volume with methanol. The differences in absorbance between 254 and 269 nm were recorded. Subsequently, the regression equation, which relates the difference between the two cited wavelengths to the drug concentration, was computed.

TLC-Densitometric Method: Calibration Curve

Aliquots equivalent to 5–45 µg from the REB standard solution (0.1 mg/mL in methanol) were transferred separately into a series of 10 mL measuring flasks. The contents of each flask were completed to volume with methanol; then 20 µL of each solution was applied in the form of bands on a TLC plate using a CAMAG Linomat IV applicator.

HPLC Method: Calibration Curve

Into a series of 10 mL volumetric flasks, aliquots equivalent to 100–1900 µg were accurately transferred from the standard stock solution of REB (0.2 mg/mL in the mobile phase), then brought to volume with mobile phase. The samples were chromatographed using the following conditions: stationary phase was a 250 × 4.6 mm 5 µm id Xterra RP C18; analytical column and the mobile phase consisted of phosphate buffer (pH 6, 0.01 M)–methanol (1 + 1, v/v). The mobile phase was filtered through a 0.45 µm Millipore membrane filter and degassed for about 15 min in an ultrasonic bath prior to use. The flow rate was 1 mL/min (isocratically at ambient temperature, 25°C), with UV detection at 254 nm. The samples were also
filtered through a 0.45 µm membrane filter and injected by a 20 µL Agilent analytical syringe. The relative peak area ratios (drug/external standard), used 110 µg/mL of REB as the external standard. Aliquots of intact drug and the degraded drug were mixed together to prepare different mixtures containing 5–90% of the degradation products; then the procedures mentioned under each method were followed. The drug concentrations were calculated from the corresponding regression equations.

Assay of Pharmaceutical Marketed Formulations (Mucosta tablets)

Ten Mucosta tablets were weighed, finely powdered, and mixed thoroughly. An amount of the powder equivalent to 0.01 and 0.02 g of REB was accurately weighed into two separate 100 mL volumetric flasks, and 50 mL of appropriate solvent was added. The mixture was sonicated for 10 min; then 25 mL solvent was added and sonicated for an additional 10 min to cause complete dissolution of REB. Volumes were completed with appropriate solvent to obtain 0.1 mg/mL and 0.2 mg/mL stock solutions, which were subsequently filtered.

Results and Discussion

The International Conference on Harmonization (ICH) Q1A (R2) guidelines require that stress testing be performed to elucidate the inherent stability characteristics of the active substance (7). An ideal stability-indicating method is one that quantifies the standard drug alone and resolves its degradation products. The present paper describes sensitive, selective, and accurate methods for determination of REB in the presence of its acid-degradation products. This information can be used in future stability studies and improved quality control applications associated with this drug.

Previously published methods of analysis for REB formulations were not validated for specificity or any degradation studies (3–6). Thus, comprehensive stress testing of REB was carried out according to the ICH guideline (7), where the drug was subjected to acid/base hydrolysis and oxidation during its forced degradation studies. The drug was stable against oxidation with 30% H₂O₂. However, while in acid and/or alkaline conditions, it was hydrolyzed through the cleavage of its amide linkage, giving rise to two pharmacologically inactive degradation products: p-chlorobenzoic acid (degradant 1) and α-amino-1,2-dihydro-2-oxo-4-quinolinepropionic acid (degradant 2). Degradant 1 is considered to be combustible, harmful, and irritating for the skin, eye, mucous membranes, and upper respiratory tract (8, 9). The suggested pathway for the drug’s degradation process is shown in Figure 1.

The structure of acid and/or alkaline degradation products was elucidated by IR and MS (Figure 2), where the IR spectrum of REB showed a characteristic NH-amide peak at 3273.5 cm⁻¹.

Table 2. Determination of REB in presence of its acid-degradation products in laboratory-prepared mixtures by the proposed methods

<table>
<thead>
<tr>
<th>Degradation products, %</th>
<th>1DD 249.4 nm</th>
<th>1DD 259 nm</th>
<th>1DD 249.4 + 259 nm</th>
<th>Dual wavelength TLC-densitometry</th>
<th>TLC-densitometry HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>—</td>
<td>99.92</td>
<td>100.02</td>
<td>99.97</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>98.90</td>
<td>99.57</td>
<td>—</td>
<td>—</td>
<td>100.83</td>
</tr>
<tr>
<td>25</td>
<td>99.80</td>
<td>99.05</td>
<td>99.54</td>
<td>100.54</td>
<td>100.83</td>
</tr>
<tr>
<td>50</td>
<td>99.36</td>
<td>98.00</td>
<td>99.62</td>
<td>99.99</td>
<td>100.99</td>
</tr>
<tr>
<td>75</td>
<td>99.50</td>
<td>99.75</td>
<td>98.08</td>
<td>99.56</td>
<td>—</td>
</tr>
<tr>
<td>90</td>
<td>99.00</td>
<td>99.20</td>
<td>99.08</td>
<td>99.56</td>
<td>100.25 ± 0.63</td>
</tr>
<tr>
<td>Mean ± RSD, %</td>
<td>99.98 ± 0.83</td>
<td>99.35 ± 0.70</td>
<td>99.45 ± 0.30</td>
<td>99.18 ± 0.77</td>
<td>99.26 ± 1.03</td>
</tr>
</tbody>
</table>
and a carbonyl-amidic peak at 1644 cm\(^{-1}\), which disappeared in the IR spectra of both degradation products. The appearance of a broad OH-carboxylic peak at 2500–3100 cm\(^{-1}\) and an acidic carbonyl peak at 1686 cm\(^{-1}\) in degradant 2 is believed to be due to the tautomerism of the zwitter ion. The mass spectra confirm these claims, where the base peak for each compound corresponds to its molecular weight.

The zero-order absorption spectra of REB and its acid degradation products show severe or even complete overlapping (Figure 3), which interferes with the direct spectrophotometric determination of REB in the presence of its degradation products.

Derivative ratio spectroscopy is an analytical technique of great utility for resolving overlapped spectra. This technique was applied successfully in the selective determination of REB in the presence of its acid degradation products by measuring the peak amplitudes at 249.4 and 259 nm and at \(1DD(249.4 + 259\text{ nm})\) (Figure 4). REB undergoes hydrolysis under acid and/or alkaline medium giving equimolar concentration of two degradation products; thus, a spectrum of their 1 + 1 mixture could be used as a divisor.

To optimize the \(1DD\) method, several divisor concentrations 5, 10, 15, and 20 µg/mL of the degradation products were tried. The best results regarding sensitivity and selectivity were obtained using 20 µg/mL of the degradation products as a divisor. Different smoothing and scaling factors were tested; a smoothing factor (\(\lambda = 4\)) and a scaling factor = 15 gave the best results in terms of sensitivity, repeatability, and S/N. Dividing the absorption spectra of REB in the range of 2–14 µg/mL by the absorption spectrum of 20 µg/mL of the acid-degradation products (as a divisor), the obtained ratio spectra were differentiated with respect to wavelength.

Linear calibration graphs were obtained for REB at 249.4 and 259 nm, and at \(1DD(249.4 + 259\text{ nm})\).

The computed regression equations were:

\[
P.A_1 = 0.0861C + 0.0451, \quad r = 0.9999, \quad 249.4\text{ nm}
\]

\[
P.A_2 = 0.0848C - 0.0014, \quad r = 0.9999, \quad 259\text{ nm}
\]

\[
P.A_3 = 0.17C + 0.0113, \quad r = 0.9999, \quad 249.4 + 259\text{ nm}
\]

where P.A1, P.A2, and P.A3 are the peak amplitudes at 249.4, 259, and 249.4–259 nm, respectively, C is the concentration of REB in µg/mL, and r is the correlation coefficient.

### Table 3. Application of the standard addition technique to the analysis of REB in tablets by several methods

<table>
<thead>
<tr>
<th>Method parameter</th>
<th>249.4 nm</th>
<th>259 nm</th>
<th>249.4 + 259 nm</th>
<th>254–269 nm</th>
<th>329 nm</th>
<th>254 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength, nm</td>
<td>249.4 nm</td>
<td>259 nm</td>
<td>249.4 + 259 nm</td>
<td>254–269 nm</td>
<td>329 nm</td>
<td>254 nm</td>
</tr>
<tr>
<td>Linearity range</td>
<td>2–14 µg/mL</td>
<td>2–14 µg/mL</td>
<td>2–14 µg/mL</td>
<td>5–65 µg/mL</td>
<td>0.50–4.5 µg/band</td>
<td>10–190 µg/mL</td>
</tr>
</tbody>
</table>

Regression equation (A = bC + a); where A is the analytical signal and C is the concentration.

<table>
<thead>
<tr>
<th>Regression equation (A = bC + a);</th>
<th>249.4 nm</th>
<th>259 nm</th>
<th>249.4 + 259 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (a)</td>
<td>0.0451</td>
<td>-0.0014</td>
<td>0.0113</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.08611</td>
<td>0.0848</td>
<td>0.17</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

### Table 4. Analytical parameters and validation results of the determination of REB by the proposed methods

<table>
<thead>
<tr>
<th>Method parameter</th>
<th>249.4 nm</th>
<th>259 nm</th>
<th>249.4 + 259 nm</th>
<th>254–269 nm</th>
<th>329 nm</th>
<th>254 nm</th>
</tr>
</thead>
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</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

Accuracy

<table>
<thead>
<tr>
<th>Mean ± RSD, %</th>
<th>99.87 ± 0.83</th>
<th>100.04 ± 0.75</th>
<th>100.28 ± 1.11</th>
<th>99.84 ± 1.06</th>
<th>99.49 ± 0.99</th>
<th>99.28 ± 0.78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity and selectivity</td>
<td>99.98 ± 0.83</td>
<td>99.35 ± 0.70</td>
<td>99.45 ± 0.30</td>
<td>99.18 ± 0.76</td>
<td>99.26 ± 1.03</td>
<td>100.25 ± 0.63</td>
</tr>
</tbody>
</table>

Precision

<table>
<thead>
<tr>
<th>RSD, %</th>
<th>±0.33</th>
<th>±0.40</th>
<th>±0.29</th>
<th>±0.39</th>
<th>±0.58</th>
<th>±0.62</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD, %</td>
<td>±0.60</td>
<td>±0.69</td>
<td>±0.60</td>
<td>±0.28</td>
<td>±1.10</td>
<td>±0.94</td>
</tr>
</tbody>
</table>

Robustness

<table>
<thead>
<tr>
<th>LOD (µg/mL)</th>
<th>0.22 µg/mL</th>
<th>0.25 µg/mL</th>
<th>0.23 µg/mL</th>
<th>1.37 µg/mL</th>
<th>0.07 µg/band</th>
<th>1.69 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ (µg/mL)</td>
<td>0.86 µg/mL</td>
<td>0.75 µg/mL</td>
<td>0.70 µg/mL</td>
<td>4.15 µg/mL</td>
<td>0.22 µg/band</td>
<td>5.12 µg/mL</td>
</tr>
</tbody>
</table>

\(a\) Recovery of REB in laboratory prepared mixtures containing up to 90% degradation products.

\(b\) Intraday precision (average of 3 different concentrations (n = 3) within the same day).

\(c\) Interday precision (average of 3 different concentrations (n = 3) repeated on 3 successive days).
the proposed method was checked by the analysis of different concentrations of pure samples in triplicate. The mean percentage recoveries were 99.87 ± 0.83, 100.04 ± 0.75, and 100.28 ± 1.11, at 249.4, 259, and 249.4–259 nm, respectively.

The second method (dual wavelength method) was simple, accurate, economical, and rapid. It uses the analytical signal data at two accurately selected wavelengths. The interference of degradation products was nullified by carefully selecting the pair of wavelengths at which the difference in REB signals had to be linear, while the difference in the degradation product’s signal remained at zero. To optimize the method, different pairs of wavelengths were selected and tried to show a zero absorbance difference for the degradation products. The difference in absorbance between REB and its degradation products without data manipulation, and can solve the problem of spectral bands overlapping between REB and its degradation products without sample pretreatment or separation steps.

The third method uses TLC-densitometry and offers a simple way to quantify REB directly on a TLC plate by measuring the optical density of the separated bands. The amounts of the unknown compounds are determined by comparing against a standard curve composed of reference materials chromatographed simultaneously under the same conditions (12). The separation allows the determination of REB with no interference from its degradation products, and the acid-degradation products (Re = 0.85) and its degradation products (Re = 0.70 and 0.65 for degradants 1 and 2, respectively).

The linearity was confirmed by plotting the integrated peak area versus the corresponding concentrations at 329 nm over a range of 0.50–4.5 µg/band, where a linear response was obtained. The TLC-scanning profile of different concentrations of REB at 329 nm is shown in Figure 5. The regression equation was:

\[ A = 0.2481C + 0.566, \ r = 0.9999 \]

where \( A \) is the integrated area under the peak \( \times 10^{-4} \) for REB, \( C \) is the concentration of REB in µg/band, and \( r \) is the correlation coefficient.

The mean percentage recovery was 99.49 ± 0.99. The precision of the proposed method was checked by the analysis of different concentrations of pure samples in triplicate.

To improve the band separation, it was necessary to investigate the effect of different variables to determine the optimum parameters.

### Mobile Phase

Different solvent developing systems of different compositions and ratios were tried for separation: methanol–ethyl acetate (9.5+0.5, v/v), chloroform–methanol (5+5, v/v), chloroform–methanol (0.5+9.5, v/v), and methanol–chloroform–acetic acid (8+2+0.1, v/v). The best-performing mobile phase was methanol–chloroform–ammonia solution (8.5+1.5+0.5, v/v). This selected mobile phase allows the determination of REB without interference from its degradation products and without tailing of the separated bands (Figure 6).

### Band Dimensions

Different band dimensions were tested to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 4 mm; the space between the bands was 14 mm.

### Scanning Wavelength

Different scanning wavelengths were tried, but the best sensitivity was obtained when REB was scanned at 329 nm, as the peaks were sharper and more symmetrical, with a minimum amount of noise.

### Slit Dimensions of Scanning Light Beam

The slit dimensions of the scanning light beam should ensure complete coverage of the band dimensions on the scanned track without interference from the adjacent bands. While different...
slit dimensions were tried, 5.0 × 0.2 mm proved to be the slit dimension of choice, which provided the highest sensitivity.

**System Suitability**

Parameters including resolution (R_s), peak symmetry, capacity factor (k’), and selectivity factor (α) were calculated. The resolution was always above two, the selectivity greater than one, and an acceptable value for symmetry factor was also obtained (Table 1).

Because the method could effectively separate the drug from its degradation products, it can be used as a stability-indicating method. The HPLC method was developed for the determination of REB in pure form and in a pharmaceutical preparation using a variety of experimental conditions, such as small changes in pH (6.0–6.5) and buffer–methanol ratio (from 1 + 1 to 1.25 + 0.75, v/v) in the mobile phase. The effect on the retention time and peak parameters was studied, and it was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified; however, the peak areas and peak symmetries were conserved.

To assess the stability-indicating efficiency of the proposed methods, the degradation products of REB were mixed with the intact sample in different ratios and analyzed by the standard addition technique (Table 3). The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions, such as small changes in pH (6.0–6.5) and buffer–methanol ratio (from 1 + 1 to 1.25 + 0.75, v/v) in the mobile phase. The effect on the retention time and peak parameters was studied, and it was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified; however, the peak areas and peak symmetries were conserved.

To optimize the proposed HPLC method, all experimental conditions were investigated. The RP methodology was selected over the normal-phase separation due to the drawbacks inherent with the normal phase, e.g., hydration of silica with water, which can cause peak tailing. Different systems were tried for chromatographic separation of the drug from its degradation products by combining homogenous design, solvent polarity optimization, and by studying the influence of the amount of organic modifier on the peak shape and retention time. The best resolution was achieved with a mobile phase consisting of phosphate buffer (pH 6, 0.01 M)–methanol (1 + 1, v/v).

**Effect of pH**

The pH of the mobile phase had a great effect on the peak shape of the separated components. Variations of the mobile phase pH resulted in a maximum capacity factor (k’) value, where at 6 < pH < 3, loss of peak symmetry was obtained.

**Instrumental Conditions**

Several wavelengths were tested. Although peaks at 329 nm gave higher sensitivity, peaks at 254 nm allowed the visualization of REB and its two degradation products. When the wavelength was greater than 255 nm, degradant 1 showed no UV absorption. In addition, the mobile phase was delivered at different rates (1, 1.5, and 2 mL/min). The optimum flow rate was 1 mL/min, which provided the maximum separation with the least run time.

**System Suitability**

System suitability parameters for the HPLC method were tested by calculating the capacity factor, tailing factor, sensitivity factor, and resolution (Table 1). The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions, such as small changes in pH (6.0–6.5) and buffer–methanol ratio (from 1 + 1 to 1.25 + 0.75, v/v) in the mobile phase. The effect on the retention time and peak parameters was studied, and it was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified; however, the peak areas and peak symmetries were conserved.

To assess the stability-indicating efficiency of the proposed methods, the degradation products of REB were mixed with the intact sample in different ratios and analyzed by the proposed methods. Table 2 illustrates good selectivity in the determination of REB in the presence of up to 90% (w/w) of its degradation products by the spectrophotometric and chromatographic methods. Also, it showed that the 1DD method had the best results regarding selectivity and precision at (1DD 259.4 nm). The suggested methods were successfully applied for the determination of REB in its pharmaceutical formulation, showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique (Table 3). The precision of the suggested methods was also expressed in terms of RSD of the interday and intraday analysis results (Table 4). Results of the suggested methods for determination of REB were statistically compared to those obtained by applying the reported HPLC method (6). As long as the variability of the values was within the acceptable range stated by ICH guidelines for accuracy and precision, the scattering of the values given in Tables 2–4 is acceptable, as shown by the %RSD and r values. The calculated t- and F-values (13) were less than the corresponding theoretical ones, confirming good accuracy and excellent precision (Table 5). To compare the ability of the proposed methods to determine pure REB in the presence of its degradation products, the obtained results were subjected to statistical analysis using

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**Table 6. ANOVA results (single factor) for comparison of the methods for the determination of REB in pure powder form**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS*</th>
<th>df</th>
<th>MS*</th>
<th>F*</th>
<th>p-value</th>
<th>F_crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>4.620</td>
<td>5</td>
<td>0.924</td>
<td>1.073</td>
<td>0.392</td>
<td>2.480</td>
</tr>
<tr>
<td>Within groups</td>
<td>31.003</td>
<td>36</td>
<td>0.861</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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* SS = Sum of squares.  
* df = degrees of freedom.  
* MS = Mean square.  
* F = Critical value corresponding to the theoretical value.
a one-way analysis of variance test. There were no significant differences between any of the proposed methods (Table 6).

Conclusions

The present work is concerned with the determination of REB in the presence of its acid degradation products where simple, sensitive, and rapid methods are described for the determination of REB in pure form or in a pharmaceutical formulation.

Upon reviewing the literature, no methods concerned with the determination of REB in the presence of its acid-degradation products could be found. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility, and specificity, and can be used as stability-indicating methods. Moreover, these methods are simple and inexpensive, thus permitting their application in quality control laboratories.

The dual wavelength method is simple, rapid, sensitive, and has no need of data manipulation. The 1DD spectrophotometric method is simple, less time-consuming, and offers economic benefits as a stability-indicating method.

The advantage of the TLC densitometric method is that several samples can be run simultaneously using only a small quantity of mobile phase. Our developed HPLC method has some advantages over the previously published methods, such as being a highly selective and quantitative stability-indicating method for the analysis of REB in the presence of its degradation products. Moreover, it is applicable over a wide range of REB concentrations and is more economical, as it saves time and uses minimal solvents; one sample can be chromatographed in about 6 ± 0.2 min.

We conclude that the proposed methods could be used as stability-indicating methods for determination of the intact drug in the presence of acid and/or alkaline degradation products, and they are suitable for the routine analysis of REB in its pharmaceutical formulation.

References

(7) ICH Q1A (R2) (2003) Stability Testing of New Drug Substances and Products; International Conference on Harmonization, IFPMA, Geneva, Switzerland