Validated spectrofluorimetric and spectrophotometric methods for the determination of brimonidine tartrate in ophthalmic solutions via derivatization with NBD-Cl. Application to stability study

F. Ibrahim, N. El-Enany, R. N. El-Shaheny* and I. E. Mikhail

ABSTRACT: Two simple, selective and accurate methods were developed and validated for the determination of brimonidine tartrate (BT) in pure state and pharmaceutical formulations. Both methods are based on the coupling of the drug with 4-chloro-7-nitro-2,1,3-benzoxadiazole in borate buffer (pH 8.5) at 70 °C and measurement of the reaction product spectrophotometrically at 407 nm (method I) or spectrofluorimetrically at 528 nm upon excitation at 460 nm (method II). The calibration graphs were rectilinear over the concentration ranges of 1.0–16.0 and 0.1–4.0 μg/mL with lower detection limits of 0.21 and 0.03, and lower quantification limits of 0.65 and 0.09 μg/mL for methods I and II, respectively. Both methods were successfully applied to the analysis of commercial ophthalmic solution with mean recovery of 99.50 ± 1.00 and 100.13 ± 0.71%, respectively. Statistical analysis of the results obtained by the proposed methods revealed good agreement with those obtained using a comparison method. The proposed spectrofluorimetric method was extended to a stability study of BT under different ICH-outlined conditions such as alkaline, acidic, oxidative and photolytic degradation. Furthermore, the kinetics of oxidative degradation of the drug was investigated and the apparent first-order reaction rate constants, half-life times and Arrhenius equation were estimated. The proposed methods are practical and valuable for routine applications in quality control laboratories for the analysis of BT. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: brimonidine tartrate; NBD-Cl; spectrophotometry; spectrofluorimetry; Stability study; ophthalmic solution

Introduction

Brimonidine tartrate (BT), chemically known as 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate, is a selective alpha-2 adrenergic receptor agonist used in the treatment of open-angle glaucoma and ocular hypertension (1). BT has a dual mechanism in lowering intra-ocular pressure; it both reduces aqueous humor production and stimulates aqueous humor outflow through the uveoscleral pathway (2).

A literature survey revealed some analytical methods for the determination of BT, including electrochemical (3), spectrophotometric (4,5), HPTLC (6), high-performance liquid chromatography (7–13), ultra-performance LC–mass spectrometry (14) and LC-MS (15) methods. The chromatographic methods always require expensive solvents in addition to elaborate treatment; meanwhile the LC-MS instrument is not available in many laboratories. On the other hand, the electrochemical method (3) for the determination of BT exhibits lower sensitivity than the present methods and requires special electrodes. Regarding the spectrophotometric methods for the determination of BT (4,5), they involved the measurement of its absorbance in the near ultraviolet region where interference most likely occurs, in addition to their lower sensitivity than the current methods. A spectrophotometric method was applied for the estimation of BT at 248 nm in phosphate buffer (pH 7.4) over the concentration range of 3.0–18.0 μg/mL (4). In addition, a first-derivative spectrophotometric method was used for BT determination over the concentration range of 2.0–12.0 μg/mL; showing maximum amplitude of the trough at 262 nm (5). A capillary electrophoresis method has also been reported for the assay of BT in aqueous humor and serum (16). A recent publication about the spectrofluorimetric determination of BT based on the measurement of its fluorescence in dimethylformamide has been reported (17). However, this method involved the use of dimethylformamide, which is a toxic and carcinogenic solvent, and is reported to damage the liver, kidneys and lungs, and cause digestive disturbances, jaundice and dermatitis upon acute and chronic exposure (18). In addition, this method (17) lacks the stability-indicating capability. Hence, our main aim was to develop new simple, safe, sensitive and specific spectrophotometric and spectrofluorimetric methods for the determination of BT in a pure state and ophthalmic solution. The study also aimed to investigate the stability of BT.

4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) is an activated aryl halide that has been used as a chromogenic and fluorogenic reagent for the determination of many drugs with primary and

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secondary amino groups (19–22). The proposed methods are based on the reaction of BT with NBD-Cl yielding a yellow-colored product that absorbed maximally at 407 nm (method I) and exhibited a strong fluorescence at 528 nm after excitation at 460 nm (method II). Method II was further applied to investigate the stability of BT under different ICH-recommended stress conditions (23,24). The proposed method appears to be a superior alternative to existing stability indicating methods (4,8,14) from economic, simplicity and sensitivity points of view.

## Experimental

### Instruments

A UV-Visible 1601 PC (P/N 206–67001) double-beam spectrophotometer (Shimadzu, Kyoto, Japan) was used for the spectrophotometric measurements. The recording range was 0–1.2. An LS 45 Luminescence Spectrometer (PerkinElmer, Buckinghamshire, UK) equipped with 150 W xenon arc lamp and 1 cm quartz cell was used for the spectrofluorimetric measurements. FL WinLab™ software was used for data acquisition. An NV P-901 digital pH-meter ( Consort, Turnhout, Belgium) was used for adjusting the pH of the buffer solutions. The photo-stability study was conducted using a UV lamp (S/N 29000), dual wavelength (254/366), 2 × 8 W (Camag, Muttenz, Switzerland).

### Materials

BT pure sample (batch no. RK128RT007) with a potency of 99.87% as determined by the comparison method (10) was kindly provided by EIPICO Co., 10th of Ramadan City-Industrial area, Egypt. Brimonidine® sterile ophthalmic solution labeled as containing 0.2% BT, batch no. NE093, product of JamJoom Pharma, Jeddah, Saudi Arabia, was purchased from a local pharmacy.

### Chemicals and reagents

All reagents were of analytical grade, solvents were of spectroscopic grade and distilled water was used throughout the study. NBD-Cl was purchased from Sigma Co. (St. Louis, USA) and freshly prepared as 0.1% (w/v) methanolic solution. Methanol and hydrochloric acid (32% w/v) were obtained from Sigma-Aldrich Co. (Munich, Germany). Sodium hydroxide, hydrogen peroxide (30% w/v) and boric acid were purchased from ADWIC Co. (Cairo, Egypt). Borate buffer solution, 0.2 M, was prepared by mixing appropriate volumes of 0.2 M boric acid and 0.2 M NaOH.

### Standard solution

A standard solution of BT containing 100 μg/mL was prepared in methanol. This solution was further diluted with the same solvent to obtain appropriate concentrations. The solution was found stable for at least 1 week when kept in a refrigerator at 4 °C.

### General procedures

#### Construction of the calibration graphs.

For method I, accurately measured volumes of BT standard solution were quantitatively transferred into a set of 10 mL volumetric flasks to obtain final concentrations of 1.0–16.0 μg/mL. Borate buffer, 1.0 mL (pH 8.5), was added followed by 1.0 mL of NBD-Cl (0.1% w/v) and solutions were mixed well. The solutions were heated in a thermostatically controlled water bath at 70 °C for 15 min. The reaction was stopped by cooling under tap water, then, 0.2 mL of 1.0 M HCl was added. The solutions were completed to the final volume with methanol and mixed well. The absorbance was measured at 407 nm against a reagent blank prepared simultaneously. For method II, the same procedure was followed using 0.8 mL of NBD-Cl and appropriate volumes of the BT standard solution to obtain a final concentration range of 0.1–4.0 μg/mL. The relative fluorescence intensity (RFI) of the reaction product was measured at 528 nm after excitation at 460 nm. The calibration graphs were constructed by plotting the absorbance (method I) or the RFI (method II) versus the final drug concentration (μg/mL) and the corresponding regression equations were derived.

#### Analysis of sterile ophthalmic solution.

Aliquot of 2.5 mL of ophthalmic solution equivalent to 5.0 mg of BT was transferred into a 50 mL volumetric flask, about 35 mL of methanol was added and the flask was sonicated for 5 min. The volume was completed with methanol and mixed well to obtain a 100 μg/mL solution. Accurately measured volumes of the solution were transferred into a series of 10 mL volumetric flasks and the procedures described under “Construction of the calibration graphs” were followed. The nominal content of the ophthalmic solution was determined either from the calibration graphs or using the corresponding regression equation.

#### Alkaline and acidic degradation.

One mL of standard solution was transferred into a 10 mL volumetric flask followed by 2.0 mL of either 1.0M NaOH or 1.0 M HCl, and the solutions were heated under reflux in a boiling water bath for 3 h. After cooling, solutions were neutralized with 1.0 M HCl or 1.0 M NaOH, respectively, completed to the final volumes with methanol and mixed well. Accurately measured volumes of these solutions were transferred into 10 mL volumetric flasks. The procedure for method II described under “Construction of the calibration graphs” was followed.

#### Photolytic degradation.

One mL of standard solution was transferred into a 10 mL volumetric flask and completed to the volume with methanol. The solution was exposed to a UV lamp at a wavelength of 254 nm at a distance of 15 cm placed in a wooden cabinet for 24 h. Accurately measured volume of this solution was transferred into a 10 mL volumetric flask and treated as described for method II under “Construction of the calibration graphs.”

#### Oxidative degradation.

For the kinetic study of the oxidative degradation, 1.0 mL of the standard solution was quantitatively transferred into a series of 10 mL volumetric flasks followed by 1.0 mL of H2O2 (3.0% w/v). Solutions were heated for increasing time intervals (5–90 min) at different temperature settings (50, 60, 70 and 80 °C) in a thermostatically controlled water bath. At the specified time, solutions were cooled, completed to volume with methanol and mixed well. Aliquots of these solutions were transferred into 10 mL volumetric flasks. The procedure described for method II under “Construction of the calibration graphs” was performed.
Results and discussion

The reaction of NBD-Cl with BT has not been investigated yet. Therefore, the present study was devoted to the investigation and application of such a reaction for the development of sensitive and simple spectrophotometric and spectrofluorimetric methods for the determination of BT in its dosage form. BT was found to react with NBD-Cl in borate buffer of pH 8.5 producing a yellow-colored adduct of maximum absorbance at 407 nm (Fig. 1a). The adduct exhibits a strong fluorescence at 528 nm after excitation at 460 nm (Fig. 1b).

Optimization of experimental conditions

Effect of pH and volume of borate buffer. The pH of borate buffer was studied over the range of 7.3–10.0. Maximum RFI was achieved at pH 8.2–8.7, and then it gradually decreased (Fig. 2). For best sensitivity, subsequent experiments were carried out at pH 8.5. Different volumes of borate buffer at pH 8.5 were then tested. It was found that using 1.0 mL of borate buffer is adequate for maximum RFI of the reaction product.

Effect of volume of 4-chloro-7-nitro-2,1,3-benzoxadiazole. The effect of the volume of NBD-Cl on the reaction rate was investigated using (0.1% w/v) solution (Fig. 3). After this study, 1.0 and 0.8 mL of NBD-Cl were chosen as the optimal volumes for methods I and II, respectively. Owing to the presence of a labile chloride in the chemical structure of NBD-Cl, a freshly prepared solution was used.

Effect of heating temperature and time. Studying the influence of heating temperature on the reaction between NBD-Cl and
BT revealed maximum RFI of the reaction product over the range of 65–75 °C. Therefore, the optimum temperature for the present study was 70 °C (Fig. 4a). Different heating times were then investigated at 70 °C. It was found that, heating for 15 min is adequate for the maximum response of the reaction product (Fig. 4b).

Effect of acidification. The RFI and the absorbance value of the decomposition product of NBD-Cl, namely, 4-hydroxy-7-nitrobenzo-2-oxa-1,2,3-diazole, which generated under the reaction conditions is quenched by decreasing the pH of the reaction. Therefore, acidification of the reaction mixture before measurement of the RFI or the absorbance remarkably decreased the background fluorescence or absorbance due to the formation of 4-hydroxy-7-nitrobenzo-2-oxa-1,2,3-diazole without affecting the drug–reagent adduct, hence the sensitivity was increased (25). The highest RFI and absorbance were achieved upon acidification with 0.2 mL of 1.0 M HCl.

Effect of diluting solvent. Dilution with different solvents such as water, methanol, ethanol, acetonitrile and dimethylformamide was attempted. The highest RFI value was achieved upon diluting with methanol (Fig. 5).

Effect of surfactants. The influence of some surfactants on the RFI of the reaction product was studied using different concentrations of sodium dodecyl sulfate and cetrimide, and they were found to exhibit a negligible effect on the RFI of the reaction product.

Effect of time on the stability of the formed reaction product. The reaction product was found to be stable for at least 1 h at room temperature as revealed by the constancy of its RFI.

Validation of the proposed methods

The validity of the proposed methods was tested regarding linearity, range, limit of quantitation (LOQ), limit of detection (LOD), accuracy, precision, robustness and specificity according to ICH Q2 (R1) recommendations (26).

Linearity and range. The calibration graphs obtained by plotting the values of the absorbance and the RFI versus the final drug concentrations (μg/mL) were rectilinear over the concentration ranges cited in Table 1. Statistical regression analysis (27) gave rise to small values of the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept ($S_a$) and the standard deviation of the slope ($S_b$) evidencing excellent linearity of the proposed methods (Table 1).

Limits of quantitation and limits of detection. The LOQ and LOD were determined according to ICH Q2 (R1) recommendation (26) adopting the following equations:

$$\text{LOQ} = 10S_a/b$$

$$\text{LOD} = 3.3S_a/b$$

Where: $S_a$ = the standard deviation of the intercept of the regression line, and $b$ = the slope of the regression line. The results are summarized in Table 1.

Accuracy. To test the accuracy of the proposed methods, they were applied to the determination of pure sample of BT over the concentration ranges cited in Table 2. Statistical evaluation of the proposed methods using the Student’s t-test and the variance ratio F-test (27) revealed no significant differences between their performance and the performance of the comparison HPLC method (10) regarding the accuracy and precision, respectively (Table 2).

Precision. The repeatability (intra-day precision) of proposed methods was tested by applying them for the determination of three concentrations of BT in pure form at three successive times within the same day (26). The intermediate (inter-day)
precision was also tested by repeated analysis of three concentrations of BT in pure form over a period of three successive days (26). The data obtained from precision study indicates high precision of the developed methods as revealed by small values of %RSD and %Error (Table 3).

Robustness. The robustness of the proposed methods was demonstrated by the constancy of the absorbance and the RFI with the deliberated minor changes in the experimental parameters. Minor intentional variation in the pH (8.5 ± 0.2), volume of buffer (1.0 ± 0.5 mL), volume of NBD-Cl (1.0 ± 0.2 mL for method I and 0.8 ± 0.2 mL for method II) and heating temperature (70 ± 5 °C) did not significantly affect the analytical response of the reaction product.

Molar ratio and mechanism of the reaction

The stoichiometry of the reaction between BT and NBD-Cl was studied adopting the limiting logarithmic method (28). Plots of log absorbance or log RFI versus log [NBD-Cl] and log [BT] gave straight lines, slopes of them were 0.7612/0.6247 and 0.7139/

Table 1. Analytical performance data for the proposed methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (μg/mL)</td>
<td>1.0–16.0</td>
<td>0.1–4.0</td>
</tr>
<tr>
<td>Limit of detection (μg/mL)</td>
<td>0.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Limit of quantitation (μg/mL)</td>
<td>0.65</td>
<td>0.09</td>
</tr>
<tr>
<td>Regression equationa (Y = a + bX) Y = 9.04 × 10⁻² + 5.33 × 10⁻² X</td>
<td>Y = 40.94 + 67.89 X</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Standard deviation of the residuals (S_y/x)</td>
<td>4.4 × 10⁻³</td>
<td>9.2 × 10⁻¹</td>
</tr>
<tr>
<td>Standard deviation of the intercept (S_a)</td>
<td>3.5 × 10⁻³</td>
<td>6.0 × 10⁻¹</td>
</tr>
<tr>
<td>Standard deviation of the slope (S_b)</td>
<td>4.0 × 10⁻⁴</td>
<td>2.9 × 10⁻¹</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.86</td>
<td>0.89</td>
</tr>
<tr>
<td>%Error (%RSD/√n)</td>
<td>0.37</td>
<td>0.40</td>
</tr>
<tr>
<td>A% (dL/g/cm)</td>
<td>5.3 × 10²</td>
<td></td>
</tr>
<tr>
<td>Molar absorptivity (L/mol/cm)</td>
<td>2.4 × 10⁴</td>
<td></td>
</tr>
<tr>
<td>Sandell’s sensitivity (μg/cm²)</td>
<td>1.89 × 10⁻³</td>
<td></td>
</tr>
</tbody>
</table>

aY = absorbance (method I) or the relative fluorescence intensity (method II); a = intercept; X = concentration of brimonidine tartrate (μg/mL) and b = slope.
0.6774 for methods I and II, respectively (Fig. 6). Hence, it was concluded that the reaction proceeds in the molar ratio of 1 : 1.

BT is an imidazoline derivative that contains the 2-amino-imidazoline group. By analogy to other 2-amino-imidazoline compounds such as clonidine (29) and tizanidine (30), BT likely exhibits tautomerism and probably exists as 2-imino-imidazolidine tautomer in crystals as well as in solution (Scheme 1a). Thus, the amino group substituent at the 2-position of imidazoline ring is always involved in tautomerism and is not available for the reaction with NBD-Cl. Accordingly, the reaction of BT with NBD-Cl occurs via the -NH of the imidazoline ring as illustrated in Scheme 1b.

Applications

**Pharmaceutical application.** The proposed methods were successfully applied to determine BT in its ophthalmic solution. The results obtained were statistically compared with those of the comparison method (10) by the Student’s t-test and the variance ratio F-test as shown in Table 2. The experimental values of t and F did not exceed the theoretical values (27), indicating the lack of significant differences between the proposed and comparison methods. Specificity and sensitivity of the proposed methods in addition to convenience and simplicity make it suitable for application in quality control laboratories.

**Stability study of brimonidine tartrate.** The proposed spectrofluorimetric method was applied to investigate the degradation behavior of BT under different stress conditions outlined by ICH guidelines (23,24). The drug was exposed to alkaline, acidic, photolysis and oxidative conditions. BT was found to be highly stable under alkaline, acidic and photolytic conditions as revealed by the constancy of the RFI values of stressed samples. On the other hand, BT was found to be labile to oxidative degradation upon heating with H2O2 solution with a consequent decrease of the RFI. The results of the stability study agreed well with those obtained by the reported ultra-performance LC-MS method (14).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I</th>
<th>Method II</th>
<th>Comparison method (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. taken (μg/mL)</td>
<td>% Found^a</td>
<td>Conc. taken (μg/mL)</td>
</tr>
<tr>
<td>Pure form</td>
<td>1.0</td>
<td>100.56</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>99.72</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>99.00</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>101.24</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>99.74</td>
<td>4.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>100.05 ± 0.86</td>
<td></td>
<td>100.08 ± 0.89</td>
</tr>
<tr>
<td>t-test</td>
<td>0.354 (2.447)^b</td>
<td></td>
<td>0.401 (2.447)^b</td>
</tr>
<tr>
<td>F-test</td>
<td>7.310 (19.247)^b</td>
<td></td>
<td>7.837 (19.247)^b</td>
</tr>
<tr>
<td>Brimonidine* sterile ophthalmic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>solution (0.2% BT)</td>
<td>4.0</td>
<td>98.40</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>99.74</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>14.0</td>
<td>100.35</td>
<td>4.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>99.50 ± 1.00</td>
<td></td>
<td>100.13 ± 0.71</td>
</tr>
<tr>
<td>t-test</td>
<td>0.196 (2.776)^b</td>
<td></td>
<td>0.806 (2.776)^b</td>
</tr>
<tr>
<td>F-test</td>
<td>1.638 (19.00)^b</td>
<td></td>
<td>1.212 (19.00)^b</td>
</tr>
</tbody>
</table>

^aEach result is the average of three separate determinations.

^bValues between parentheses are the tabulated t and F values at P = 0.05 (27).

### Table 3. Precision data for the determination of brimonidine tartrate in pure form by the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>intra-day precision</th>
<th>inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. taken (μg/mL)</td>
<td>Mean % found ± SD</td>
</tr>
<tr>
<td>I</td>
<td>4.0</td>
<td>99.41 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>100.74 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>99.94 ± 0.30</td>
</tr>
<tr>
<td>II</td>
<td>0.2</td>
<td>100.58 ± 1.95</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>100.94 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>98.36 ± 0.37</td>
</tr>
</tbody>
</table>
kinetics and to be temperature dependent (Fig. 7a). The apparent first-order degradation rate constant \( (k) \) and the half-life time \( (t_{1/2}) \) were calculated at each temperature (Table 4). The Arrhenius plot was constructed by plotting \( \log k \) values versus \( 1/T \) (Fig. 7b).

Arrhenius equation (31) was derived and it was found to be:

\[
\log k = \frac{1.093 \cdot (1.328/T)}{T}.
\]

Where, \( T \) is the absolute temperature (\( °K = 273 + °C \)).

The activation energy \( (E_a) \) for the oxidative degradation of BT was calculated from the slope of Arrhenius plot (31) and found to be 6.08 kcal/mol.

The imidazoline ring of BT contains the \(-\text{NH}\) group, which is probably labile to oxidation with hydrogen peroxide (32). Therefore, it is suggested that the degradation pathway is via the formation of N-oxide derivative (33) (Scheme 2). The formation of BT, brimonidine tartrate; NBD-Cl, 4-chloro-7-nitro-2,1,3-benzoxadiazole; RFI, relative fluorescence intensity.

### Table 4. Effect of temperature on the kinetic parameters of brimonidine tartrate

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Reaction rate constant ( (k \times 10^{-3}, \text{min}^{-1}) )</th>
<th>Half-life time ( (t_{1/2}, \text{h}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.97</td>
<td>11.9</td>
</tr>
<tr>
<td>60</td>
<td>1.20</td>
<td>9.6</td>
</tr>
<tr>
<td>70</td>
<td>1.79</td>
<td>6.5</td>
</tr>
<tr>
<td>80</td>
<td>2.10</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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**Scheme 1.** (a) Tautomerism of brimonidine tartrate and (b) proposed reaction pathway of brimonidine tartrate with NBD-Cl. NBD-Cl, 4-chloro-7-nitro-2,1,3-benzoxadiazole.
N-oxide BT derivative resulted in blocking the coupling site for the reaction with NBD-Cl. Hence, the proposed method is considered a stability-indicating assay for the drug in the presence of its oxidative degradation product where the latter did not react with NBD-Cl.

**Conclusion**

The proposed spectrophotometric and spectrofluorimetric methods provided sensitive, specific, safe and inexpensive analytical procedures for the determination of BT either in pure form or in its ophthalmic dosage forms without interference from common excipients. Moreover, these methods are less time-consuming and do not require elaborate treatments associated with chromatographic methods. The spectrofluorimetric method was applied in the BT stability study under different ICH recommended conditions; hence, it provides a stability-indicating tool for rapid screening of BT stability in pure form and eye drops. These attributes, in addition to the satisfactory sensitivity and reproducibility as well as the convenience and simplicity, make the proposed methods suitable for routine analysis in quality control laboratories.

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