Scheme 1: Proposal of the reaction pathway between **Los** and **BTB**.

Bromothymol Blue   Losartan

in chloroform

Losartan-Bromothymol blue complex
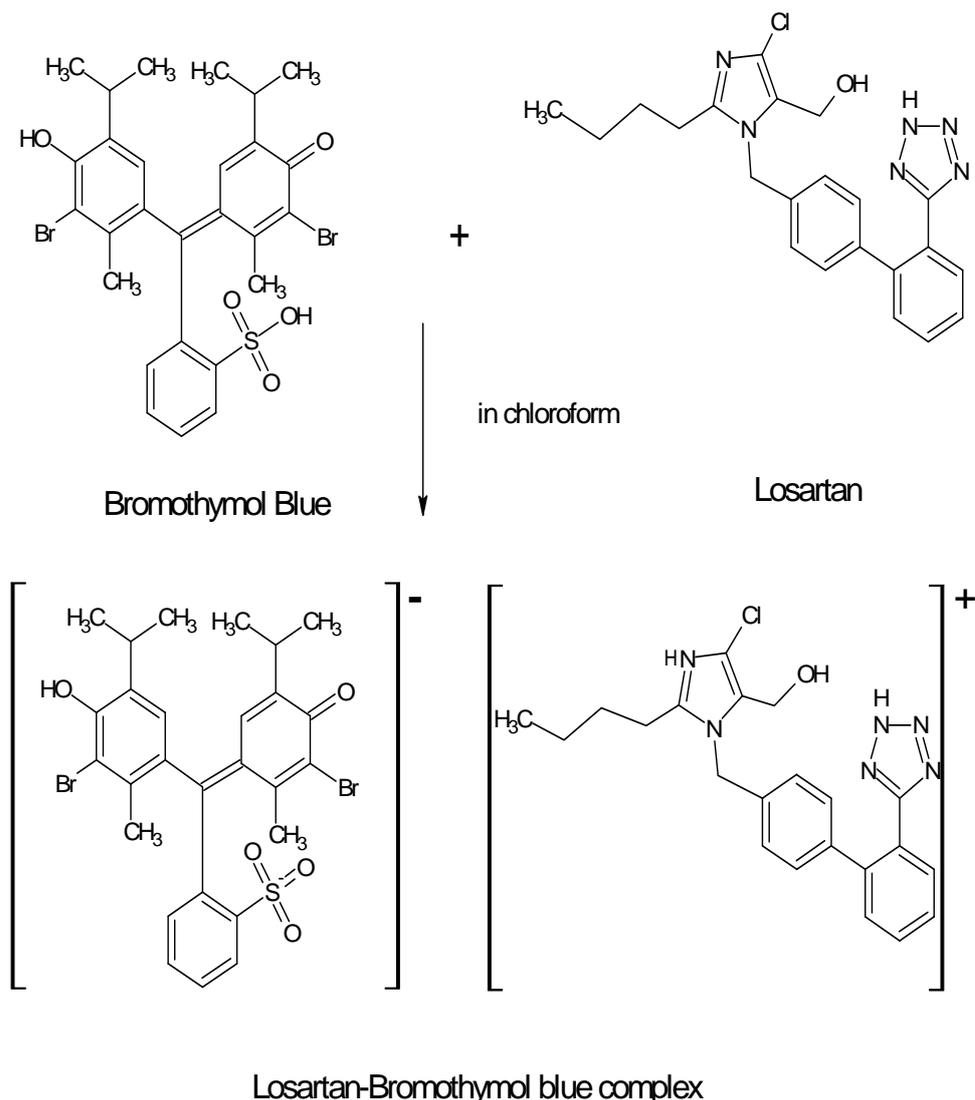
4. Validation of the proposed methods:
4.3.1. Linearity of the method

Under the above experimental conditions, The calibration curves were constructed by plotting concentration versus absorbance or ΔRFI for spectrophotometric and spectrofluorimetric methods respectively.

The statistical parameters were calculated from regression equation drived from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation of residuals ($S_{y/x}$) with good correlation coefficients and small intercepts. The apparent molar absorptivities and detection limits [46] were summarized in (Table 1).

4.3.2. Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed methods were calculated using the following equations [47, 48]
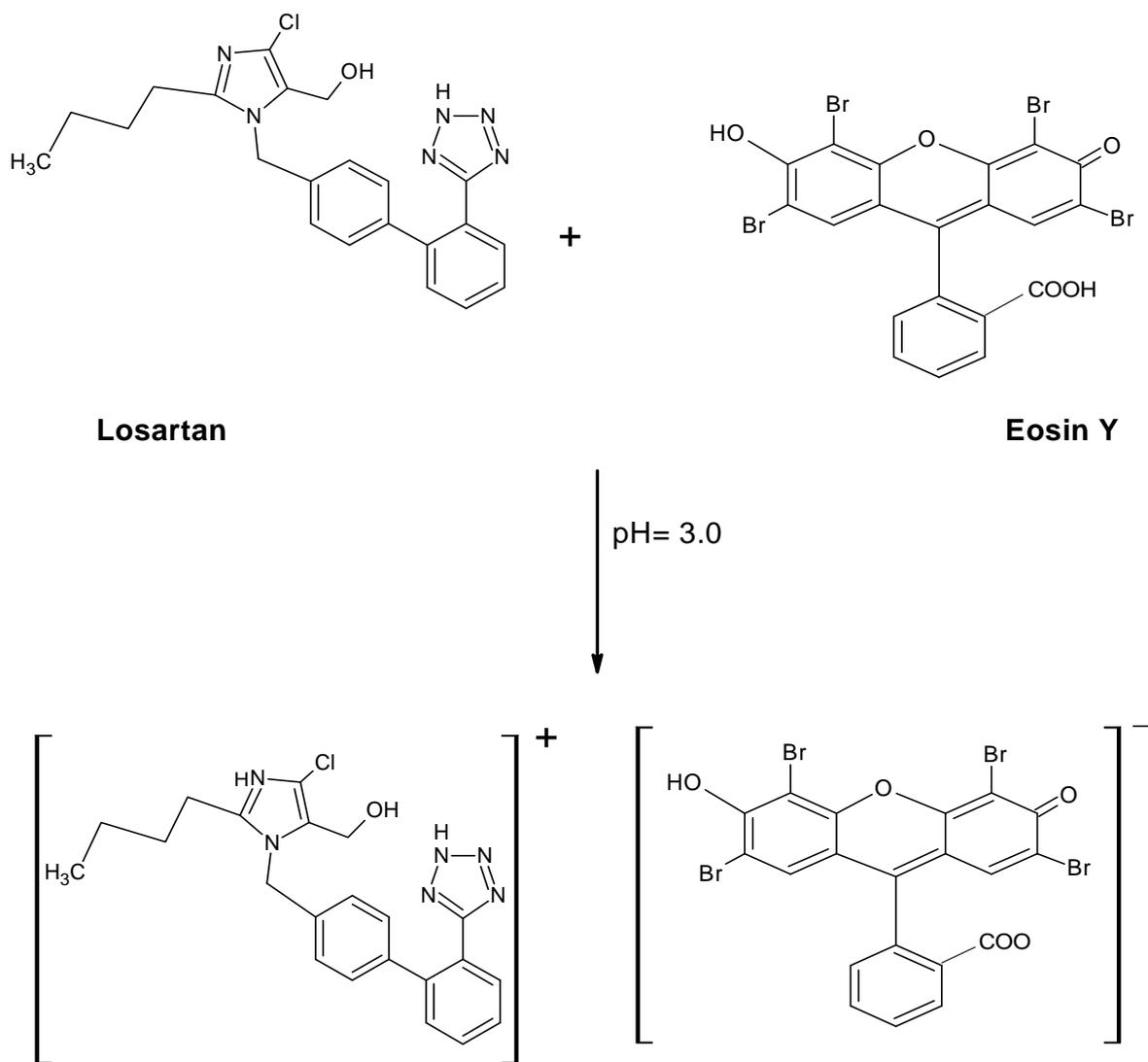
LOD   =   3.3   σ/s

LOQ = 10 σ/s

σ is the standard deviation of intercept, **s** is the slope of calibration curve.

LOQs and LODs for the studied drugs are listed in (Table 1).

Scheme 2: Suggested reaction mechanism for reaction between **Los** and **Eosin Y**.

Losartan   **Eosin Y**

pH= 3.0

Losartan - Eosin Y ion pair associates

4.3.3. Specificity and interference

The specificity of the proposed methods was investigated by observing any interference encountered from common excipients in the pharmaceutical tablets such as starch, magnesium stearate, and Talc. It was found that these excipients did not interfere with the results of the proposed methods. This could be explained on the basis that all these additives are either non extractable by organic solvents or behave as non basic compounds, so they do not contribute in the reaction pathway.

4.3.4. Precision and accuracy

Statistical analysis of the regression equations allowed the calculation of standard deviation of the intercept (S_a), standard deviation of the slope (S_b) and standard deviation of the residuals ($S_{y/x}$). The small values of these parameters indicate the high precision of the proposed methods [46]. In order to determine the accuracy and the precision of the method, the accuracy was checked by

three times analysis for five different concentrations of pure samples. The results obtained in (Table 3) showed the close agreement between the measured and true values indicating good accuracy of the proposed method . Intraday and Interday precision were assessed using three concentration and three replicates of each concentration. The calculated relative standard deviation values were found to be small below 2 % indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis were summarized in (Table 3).

4.3.5. Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged such as dye concentration for the spectrophotometric method, pH and buffer volume for spectrofluorimetric method. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D. % of the same procedure applied by using two different instruments on different days. The results showed no statistical differences between different instruments suggesting that the developed methods were robust and Spectrophotometric method was rugged.

5. Application to pharmaceutical tablets

The proposed methods have been successfully applied to the determination of the studied drugs in commercial tablets.
The results obtained are shown in Table (3). Six replicate determinations were made. Table (4) shows that satisfactory recovery data were obtained and the assay results were in a good agreement with the label claims. On comparison of the results obtained by the proposed methods with those of the reported methods [11, 33, 49, 50]

using the t-test for the accuracy and F-test for the precision assessment, the calculated values did not exceed the corresponding theoretical values (tabulated value of t-test and F-test is at confidence level 95% = 6.39 and 2.78 for n = 5 degrees of freedom respectively) indicating no significant difference between proposed and reported methods.

6. Conclusion:

The proposed methods are rapid, simple, accurate, and extraction-free for the analysis of certain ARBs in their pure forms and tablets. Compared with the reported methods, the proposed methods are very simple, requiring only one reagent and non expensive instrumentation. The lower quantitation limit of the proposed method is much lower ($1.0\mu g$ mL^{-1}). These advantages encourage the application of the proposed methods in routine quality control evaluation.

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