Validated Spectrophotometric Method for Determination of Some Benzimidazole Derivatives in Pharmaceutical Formulations Using 1,2-naphthoquinone-4-sulphonate

Safwan Ashour¹* and Roula Bayram¹

¹Department of Chemistry, Faculty of Sciences, University of Aleppo, Aleppo, Syria.

ABSTRACT

**Aim:** To develop and validate a sensitive method for the determination of rapéprazole (RPZ) and omeprazole (OPZ) in bulk and formulations based on nucleophilic substitution reaction of RPZ and OPZ with sodium 1,2-naphthoquinone-4-sulphonate (NQS) in an alkaline medium.

**Study Design:** All variables were studied to optimize the reaction conditions and the reaction mechanism was postulated.

**Place and Duration of Study:** Department of Chemistry, Faculty of Science, Aleppo University, Aleppo, Syria between December 2011 and December 2012.

**Methodology:** The colored products were measured spectrophotometrically at 453 nm using double beam UVD-2960 (Labomed, INC., U.S.A) ultraviolet-visible spectrophotometer with matched 1-cm quartz cells. The reaction time and temperature were 20 min and 25°C for RPZ and OPZ. As per ICH guidelines, the proposed method was validated. The developed method was successfully applied for the estimation of RPZ and OPZ in tablets and capsules and results were compared statistically with the official methods.

*Corresponding author: Email: profashour2010@myway.com;
**Results:** The developed method showed a linear Beer's law range from 0.26 to 12.0 and from 0.49 to 12.0 μg mL\(^{-1}\) with limit of detection values of 0.181 and 0.187 μg mL\(^{-1}\) for RPZ and OPZ, respectively. The calculated molar absorptivity values are 7.7×10\(^4\) and 3.8×10\(^4\) L mol\(^{-1}\) cm\(^{-1}\) for RPZ-NQS and OPZ-NQS, respectively. The proposed methods were successfully applied to the determination of RPZ and OPZ in formulations and the results tallied well with the label claim.

**Conclusion:** The developed method was linear, sensitive, selective, precise, accurate and robust, being suitable for routine quality control analyses of RPZ and OPZ.

**Keywords:** Rabeprazole; omeprazole; sodium 1,2-napthoquinone-4-sulfonate (NQS); spectrophotometry; pharmaceutical formulations.

### 1. INTRODUCTION

Rabeprazole, \(2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H benzimidazole\) and omeprazole, \(5-Methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl]methyl]sulfanyl]-1H-benzimidazole\) are an irreversible proton pump (H\(^+\)/K\(^+\)-ATPase) inhibitor (PPI) that decrease acid secretion from gastric parietal cells [1,2]. They are also effective in Zollinger-Ellison syndrome and preventing ulcer rebleeding. Thus, rabeprazole and omeprazole are valuable alternate to other PPI in the treatment of acid-related disorders [2,3]. Several methods have been described for the quantitative determination of rabeprazole and omeprazole by high-performance liquid chromatography (HPLC) in different pharmaceutical preparations, either alone [4-8] or with other active ingredients [9-11]. HPTLC [11-13], capillary electrophoresis [14-16], voltammetric [17,18] and polarographic [19] methods have been developed for the analysis of rabeprazole and omeprazole from its individual and combined formulations with other active ingredients. Various spectrophotometric methods have been reported for the determination of rabeprazole [7,20-26] and omeprazole [8] from its individual and combined formulations with other active ingredients. Derivative spectrophotometric methods are available for the simultaneous determination of rabeprazole [27,28] and omeprazole [29] with other active ingredients in dosage forms. An increasing number of publications are appearing describing the development of methods for rabeprazole and omeprazole determination in biological fluid samples [30–35]. The official procedures in pharmaceutical preparations utilize high performance liquid chromatography [36] and potentiometry [37] for rabeprazole and omeprazole, respectively. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost and wide availability in quality control laboratories. Therefore, the development and validation of a new alternative spectrophotometric method for the determination of rabeprazole and omeprazole in pharmaceutical preparations that can overcome the disadvantages of the existing methods is essential.

1,2-napthoquinone-4-sulphonate (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines. It is a popular spectrophotometric reagent due to its efficient reactivity with both primary and secondary amines, and high reaction rate [38,39]. NQS proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group, however the use of NQS for spectrophotometric determination of rabeprazole and omeprazole was not reported. Therefore, the present work describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for determining the content of rabeprazole and omeprazole in
pharmaceutical formulations based on the reaction of NQS with amino group of rabeprazole and omeprazole molecules to form orange compounds.

2. EXPERIMENTALS

2.1 Apparatus

Double beam UVD-2960 (Labomed, INC., U.S.A) ultraviolet-visible spectrophotometer with matched 1-cm quartz cells was used for all the spectrophotometric measurements under the following operating conditions: scan speed medium (400nm/min), scan range 400-600 nm and slit width 0.1 nm. Spectra were automatically obtained by UV-WIN software Ver.5.0.10. Electronic balance (Kern, Germany) was used for weighing the samples and semiautomatic micropipettes were used for measuring the volume of samples.

2.2 Materials and Reagents

Working reference standards of rabeprazole (RPZ) was supplied by Nifty Lab (India), its purity was 100.5% [36], and omeprazole (OPZ) was supplied by SL Drugs & Pharmaceuticals (India), its purity was 99.41% [37]. Sodium 1,2-naphthoquinone-4-sulphonate (NQS), from Aldrich Chemical Co., St. Louis (USA) and methanol was purchased from Labscan (Ireland). All reagents were of analytical reagent grade. Double distilled water was used in all experiments.

2.3 Pharmaceutical Formulations

The following commercial formulations were subjected to the analytical procedures:

1. Rabiral tablets (BPI, Syria) labeled to contain 10mg rabeprazole/tablet.
2. Rabiral tablets (BPI, Syria) labeled to contain 20mg rabeprazole/tablet.
3. Newprazole tablets (Oubari Pharma, Syria) labeled to contain 20mg rabeprazole/tablet.
4. Rabemax tablets (City Pharma, Syria) labeled to contain 20mg rabeprazole/tablet.
5. Omepral capsules (Asia, Syria) labeled to contain 20mg omeprazole/capsule.
6. Omeprazole capsules (Oubari Pharma, Syria) labeled to contain 20mg omeprazole/capsule.

2.4 Solutions

2.4.1 Stock standard solutions

An accurately weighed 0.05 g standard sample of RPZ and OPZ was dissolved in methanol, transferred into a 100 mL standard flask and diluted to the mark with methanol to obtain 0.5 mg mL\(^{-1}\). This stock solution was further diluted to obtain working solutions in the ranges of 0.26-12.00 and 0.49-12.00 \(\mu\)g mL\(^{-1}\) for RPZ and OPZ, respectively.

2.4.2 Sodium 1,2-naphthoquinone-4-sulfonate solution

An accurately weighed 0.3 g of NQS was dissolved in double distilled water, transferred into a 100 ml standard flask and diluted to the mark with double distilled water and mixed well to prepare 0.3% w/v. The solution was freshly prepared and protected from light during use.
2.4.3 Alkaline solutions

Sodium hydroxide, disodium hydrogen phosphate, borax and sodium bicarbonate solutions of a concentration range of 0.1-0.6 M were prepared in double distilled water.

2.5 General Procedures

Aliquots of standard RPZ (5-240 µL, 0.5 mg mL⁻¹) and OPZ (9-240 µL, 0.5 mg mL⁻¹) solutions were transferred into a series of 10mL calibrated volumetric flasks. Then 1.75 mL and 2.0 mL of 0.2 M sodium hydroxide solution was added, followed by 1.0 mL and 1.75 mL of NQS 0.3% (w/v) for RPZ and OPZ, respectively, and then the solutions were allowed to proceed at 25°C for 20 min for RPZ and OPZ. After that, the volume was made up to the mark with bidistilled water and the absorbance was measured at 453 nm for RPZ-NQS and OPZ-NQS against reagent blank treated similarly under identical conditions.

2.6 Procedures for Formulations

2.6.1 Tablet (or capsule) sample solutions

Twenty tablets were weighted accurately and crushed to a fine powder. In the case of capsules, the contents of twenty capsules were completely evacuated from shells. An accurately weighed quantity of the powder equivalent to 50 mg of rabeprazole or omeprazole was transferred into a 100 mL calibrated flasks, and dissolved in about 50mL of methanol. The contents of the flask were swirled for 10 min, and then completed to volume with methanol to achieve a concentration of 0.5 mg mL⁻¹. The contents were mixed well and filtered rejecting the first portion of the filtrate. The general procedure was then followed in the concentration ranges mentioned above.

3. RESULTS AND DISCUSSION

RPZ and OPZ were found to react with NQS in an alkaline medium at 25°C producing an orange-colored product of maximum absorption peak (λ_max) at 453 nm (Fig. 1). Thus, this wavelength was chosen for all further measurements in order to obtain highest sensitivity for the method. It is important to point out that the colorless reagent blank (NQS), in alkaline medium, exhibits negligible absorption at 453 nm. Under the experimental conditions pure drug showed a negligible absorbance at the corresponding maximum.
Fig. 1. Absorption spectra of solutions of (A,B) RPZ and OPZ (10 and 7 µg mL$^{-1}$, respectively) against methanol, (C) reagent blank against distilled water, (D) OPZ (7 µg mL$^{-1}$) and (E) RPZ (10 µg mL$^{-1}$) with NQS (0.3% w/v) against reagent blank

3.1 Optimization of Reaction Variables

Optimum conditions necessary for rapid and quantitative formation of colored product with maximum stability and sensitivity were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. In order to establish the experimental conditions, the effect of various parameters such as volume of NQS, addition of alkaline medium, waiting time and the stability of colored product were studied.

3.1.1 Effect of the solvent nature

The solvent plays an important role in some charge transfer reactions, since it must be able to facilitate the total charge transfer and then allow the complex dissociation and stabilization of the radical anion formed, which is the absorbing specie. The reaction was tested in water, methanol, ethanol, isopropanol, acetone and acetonitrile media. According to the literature, solvents with high dielectric constant are more effective to execute this task. Taking this fact into account and the high solubility of the NQS in water allow its use in the present case. Although the highest dielectric constant of acetonitrile, best sensitivity was achieved with water, probably because of the capacity of this solvent to form stable hydrogen bonds with the radical anion. Then, water was chosen for further experiments.

Maximum absorbance of the orange solutions produced by the reaction between RPZ or OPZ with NQS against reagent blank was observed at 453 nm in water medium (Fig. 1). Thus, this wavelength was chosen for all further measurements in order to obtain highest
sensitivity for the method. It is important to point out that the colorless reagent blank (NQS), in water medium, exhibits negligible absorption at 453 nm.

### 3.1.2 Effect of the NQS volume

The maximum conversion of the analyte into absorbing specie depends on the amount of the reagent available in the solution for reaction and the equilibrium involved. So, the reagent concentration in solution was studied by varying the NQS volume in the range of 0.25 - 3.0 mL of 0.3% (w/v) NQS, while the RPZ and OPZ concentration was maintained constant at 12 and 8 μg mL⁻¹, respectively. The results are shown in the Fig. 2. The study revealed that the reaction was dependent on NQS reagent. The highest absorption was attained when the volume of NQS was 1.75 and 2 mL of 0.3% (w/v) NQS for RPZ and OPZ (Fig. 2). Higher volume of NQS had no effect on the absorption values.

![Absorbance vs Volume of NQS](image)

**Fig. 2.** Effect of volume of NQS (0.3% w/v) on the reaction of RPZ (12 μg mL⁻¹) and OPZ (8 μg mL⁻¹) with NQS; NaOH 0.2 M: 1 and 0.75 mL for RPZ and OPZ, respectively; temperature: 25±5 °C; reaction time: 20 min

### 3.1.3 Effect of the alkalinity

To generate the nucleophile from RPZ or OPZ and activate the nucleophilic substitution reaction, alkaline medium was necessary; since the results revealed that RPZ or OPZ have difficulty to react with NQS in acidic media. Different inorganic bases were tested: sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solution. Best results were obtained in case of sodium hydroxide where with other bases either precipitation of white colloid occurred upon diluting the reaction solution with organic solvent, high blank readings, non reproducible results, and/or weak sensitivity were observed. Studies for optimization of sodium hydroxide concentration revealed that the optimum volume of NaOH was 1 and 0.75 mL of 0.2 M NaOH for RPZ and OPZ, respectively (Fig. 3). At this value, the amino group of RPZ and OPZ facilitates the nucleophilic substitution reaction. At more concentrations of NaOH, the absorbance of solution obviously decreased. This was attributed probably to the increase in the amount of hydroxide ion that holds back the condensation reaction between RPZ or OPZ and NQS.
3.1.4 Effect of the reaction temperature and time

The effect of temperature and time on the reaction of RPZ and OPZ with NQS in alkaline medium was studied at different values (20-75 °C, 0-60 min) by continuous monitoring of the absorbance at 453 nm. It was found that the reaction with NQS was not affected by increasing the temperature, and the reaction at laboratory ambient temperature (25±5 °C) went to completion within 20 min. The results revealed that increasing the temperature had negative effect on the absorption values of the reaction solution. This was probably attributed to the instability of the RPZ-NQS and OPZ-NQS derivative. The optimum reaction time was determined at laboratory ambient temperature. Increase absorbance values were observed from the beginning of the experiment up to 20 min (Fig. 4). After this time and up to 60 min, absorbance suffered a slight increase, reaching values up to 2% higher than those observed after 20 min of the reaction. In view of these results, all measurements were carried out after 20 min of mixing of the reagents in order to make the method faster.

Fig. 3. Effect of concentration of 0.2 M NaOH in the presence of 1.75 and 2 mL of 0.3% NQS for RPZ and OPZ, respectively, on the formation of colored products drug-NQS. RPZ (12 μg mL⁻¹) and OPZ (8 μg mL⁻¹); temperature: 25±5 °C; reaction time: 20 min for RPZ and OPZ.

Fig. 4. Effect of temperature and time on the reaction of RPZ (12 μg mL⁻¹) and OPZ (8 μg mL⁻¹) with NQS; NaOH 0.2M: 1 and 0.75 mL; NQS 0.3%: 1.75 and 2 mL for RPZ and OPZ, respectively.
3.2 Stoichiometric Relationship

Under the optimum conditions, the stoichiometry of the reaction between benzimidazole derivatives RPZ or OPZ and NQS was investigated by Job's method of continuous variation [40]. The stoichiometric ratio between NQS and drug was found to be 1:1 (Fig. 5).

Based on this ratio, and the presence of only one center (N–H group) in RPZ or OPZ molecule that is available for the nucleophilic substitution reaction, the reaction pathway was postulated to be proceeded as shown in Fig. 6. The formation constant $\beta_n$ of the formed complex is calculated using the data of the continuous variation method by applying Harvey and Manning method [41]:

$$\beta_n = \frac{A / A_{\text{max}}}{[1 - (A / A_{\text{max}})]^{n+1} C_L n^2}$$

Where $A$ is the absorbance value of the formed complex in the presence of dye concentration $C_L$; $A_{\text{max}}$ is the maximum absorbance value in the presence of excess dye concentration and $n$ is stoichiometric ratio (drug:dye). Logarithmic formation constant is summarized in Table 1.
3.3 Analytical Methods Validation

3.3.1 Linearity, limits of detection and quantification

In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance at appropriate wave lengths of a set of solutions containing varying amounts of RPZ or OPZ and a specified amount of reagent (as given in the recommended procedures for each drug) in aqueous alkaline medium were recorded against the corresponding reagent blank. The Beer's law plots of these systems are recorded graphically. Beer's law range, molar absorptivity, Sandell's sensitivity and Ringbom optimum concentration range for RPZ and OPZ were calculated. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values. The results are summarized in Table 1. In the proposed methods, linear plots with good correlation coefficients (more than 0.999) were obtained in the concentration ranges of 0.26–12.0 and 0.49–12.0 μg mL⁻¹ for the RPZ and OPZ, respectively (Table 1). The minimum level at which the investigated compound can be reliably detected (limit of detection LOD) and quantified (limit of quantification, LOQ) were determined experimentally. The detection limit for the proposed method was calculated by using the following relationship [42]:

\[
\text{Detection limit} = \sqrt{\frac{S_0^2}{\frac{n - 2}{n - 1} b} \frac{n - 2}{n - 1} t}
\]

Where \( n \) is the number of the samples; \( b \) the slope of line of regression; \( t \) is the student's t-value at 95% confidence level; \( S_0^2 = \text{Variance} = \sum (A - A_{\text{calc.}})^2 / n - 2 \) [43] (A is the
The experimental value of absorbance; $A_{\text{calc}}$ is the absorbance value calculated from the regression equation).

Limits of detection for RPZ and OPZ were found to be 0.181 and 0.187 $\mu$g mL$^{-1}$, respectively. The limit of quantification (LOQ) was determined as the lowest concentration of investigated compound used in the construction of the corresponding standard curve and defined as 0.26 and 0.49 $\mu$g mL$^{-1}$ for RPZ and OPZ, respectively.

### Table 1. Optical characteristics and statistical data using the proposed method

| Parameters                                      | RPZ          | OPZ          |
|------------------------------------------------|--------------|--------------|
| Color                                           | Orange       | Orange       |
| $\lambda_{\text{max}}$ (nm)                    | 453          | 453          |
| Formation time (min)                            | 20           | 20           |
| Stability (hr)                                  | 24           | 24           |
| Stoichiometric relationship, drug:NQS           | 1:1          | 1:1          |
| Logarithmic formation constant                  | 4.92         | 5.21         |
| Beer's law range ($\mu$g mL$^{-1}$)             | 0.26-12.0    | 0.49-12.0    |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)     | $7.7\times10^4$ | $3.8\times10^4$ |
| Ringbom optimum range ($\mu$g mL$^{-1}$)       | 1.5-10.0     | 1.18-5.06    |
| Detection limit ($\mu$g mL$^{-1}$)              | 0.181        | 0.187        |
| Sandell's sensitivity ($\mu$g cm$^2$ per 0.001 absorbance unit) | 0.009        | 0.018        |
| Regression equation                             |              |              |
| Slope ($m$)                                     | 0.1213       | 0.0686       |
| Intercept ($b$)                                  | 0.0972       | 0.0745       |
| Correlation coefficient ($r$)                    | 0.9999       | 0.9999       |
| Mean % recovery ± S.D.                          | 100.78±0.44  | 101.34±0.35  |

* $A = mC + b$, where, $C$ is the concentration of drug ($\mu$g mL$^{-1}$) and $A$ is absorbance unit.

### 3.3.2 Accuracy and precision

The accuracy and precision of the proposed methods were carried out by six determinations at different concentrations and compared with the official methods for RPZ [36] and OPZ [37]. Percentage relative standard deviation (RSD%) as precision and percentage relative error (Er%) as accuracy of the suggested and official methods were calculated. Table 2 shows the values of RSD% Er% for different concentrations of the drugs determined from the calibration curves. These results are of accuracy and precision show that the proposed method has good repeatability and reproducibility. The values of $t$- and $F$-tests obtained at 95% confidence level and five degrees of freedom [44] did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The proposed method was found to be selective for the estimation of drug in the presence of various tablet excipients. For this purpose, a powder blend using typical tablet excipients was prepared along with the drug and then analyzed. The recoveries were not affected by the excipients and the excipients blend did not show any absorption in the range of analysis.
Table 2. Accuracy and precision for the determination of RPZ and OPZ in bulk powder by the proposed method

| Drug | Taken µg mL⁻¹ | Proposed method | Official method |
|------|---------------|----------------|----------------|
|      | Mean±S.D. (µg mL⁻¹) | RSD % | Er% | t-test | Mean±S.D. (µg mL⁻¹) | RSD % | Er% | t-test |
| RPZ  |               |         |     |       |               |         |     |       |
| 2.00 | 1.99±0.06     | 3.39    | -0.50 | 1.67 | 2.01±0.07 | 3.48    | 0.50 | 1.95 | 1.36 |
| 4.00 | 4.06±0.08     | 1.95    | 1.50  | 1.94 | 4.08±0.09 | 2.20    | 2.00 | 2.05 | 1.26 |
| 8.00 | 8.11±0.07     | 0.83    | 1.37  | 1.59 | 8.09±0.10 | 1.24    | 1.12 | 1.77 | 2.04 |
| 12.00| 12.09±0.04    | 0.35    | 0.75  | 1.34 | 12.05±0.06 | 0.66    | 0.42 | 1.82 | 2.25 |
| OPZ  |               |         |     |       |               |         |     |       |
| 2.00 | 2.02±0.06     | 2.94    | 1.00  | 1.78 | 1.98±0.05 | 2.52    | -1.00| 2.01 | 1.44 |
| 4.00 | 4.11±0.09     | 2.05    | 2.75  | 2.02 | 4.07±0.08 | 1.96    | 1.75 | 1.59 | 1.27 |
| 8.00 | 8.09±0.07     | 0.90    | 1.13  | 1.83 | 8.12±0.06 | 0.74    | 1.50 | 1.79 | 1.36 |
| 12.00| 12.06±0.06    | 0.49    | 0.50  | 1.53 | 12.14±0.05 | 0.41    | 1.16 | 1.62 | 1.44 |

*Six independent analyses.

bTheoretical values for t and F-values at five degree of freedom and 95% confidence limit are (t = 2.776) and (F = 6.26).

3.4 Application to the Pharmaceutical Dosage Forms

The performance of the proposed methods was assessed by comparison with the reference methods for RPZ [36] and OPZ [37]. Mean values were obtained with a Student’s t- and F-tests at 95% confidence limits for four degrees of freedom [44]. The results showed comparable accuracy (t-test) and precision (F-test), since the calculated values of t- and F-tests were less than the theoretical data. The proposed procedures were applied to determine the studied drugs in their pharmaceutical formulations. The results in Table 3 indicate the high accuracy and precision. As can be seen from Table 3, the proposed method has the advantages of being virtually free from interferences by excipients such as glucose, lactose, and starch or from common degradation products. The results obtained were compared statistically by the student’s t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the reference methods on samples of the same batch (Table 3). The values of t- and F-tests obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared.
Table 3. Application of the proposed method to the determination of RPZ and OPZ drugs in dosage forms

| Sample                                | % Recovery ± S.D.                  | Official method |
|---------------------------------------|-----------------------------------|-----------------|
|                                       | Proposed method                   |                 |
| Pure RPZ                              | 100.78 ± 0.44                     | 100.10 ± 0.30   |
| t-value                               | 1.75                              |                 |
| F-value                                | 2.15                              |                 |
| Rabiral 10 tablets (20mg RPZ/tablet)  |                                   |                 |
| X ± S.D.                               | 99.60 ± 0.97                      | 100.19 ± 1.03   |
| t-value                                | 1.35                              | 2.00            |
| F-value                                | 1.13                              |                 |
| Rabiral 20 tablets (20mg RPZ/tablet)  |                                   |                 |
| X ± S.D.                               | 100.52 ± 1.13                     | 101.05 ± 1.01   |
| t-value                                | 2.18                              | 2.03            |
| F-value                                | 1.25                              |                 |
| Newprazole tablets (20mg RPZ/tablet)  |                                   |                 |
| X ± S.D.                               | 102.30 ± 0.69                     | 100.35 ± 0.89   |
| t-value                                | 1.92                              | 2.05            |
| F-value                                | 1.66                              |                 |
| Rabemax tablets (20mg RPZ/tablet)     |                                   |                 |
| X ± S.D.                               | 99.95 ± 0.89                      | 102.00 ± 1.10   |
| t-value                                | 1.07                              | 1.38            |
| F-value                                | 1.53                              |                 |
| Pure OPZ                               | 101.34 ± 0.35                     | 101.65 ± 0.24   |
| t-value                                | 2.04                              |                 |
| F-value                                | 2.13                              |                 |
| Omepral capsules (20mg OPZ/capsule)   |                                   |                 |
| X ± S.D.                               | 101.76 ± 0.43                     | 100.33 ± 0.39   |
| t-value                                | 1.92                              | 2.06            |
| F-value                                | 1.22                              |                 |
| Omeprazole capsules (20mg OPZ/capsule)|                                   |                 |
| X ± S.D.                               | 100.18 ± 0.48                     | 100.04 ± 0.40   |
| t-value                                | 1.84                              | 1.77            |
| F-value                                | 1.44                              |                 |

*Five independent analyses.

bTheoretical values for t and F-values at four degree of freedom and 95% confidence limit are (t = 2.776) and (F = 6.26).

4. CONCLUSION

The substitution reaction between RPZ or OPZ and NQS as a chromogenic reagent has been investigated. The obtained complex was studied by visible spectrophotometry. The obtained colored complex was utilized in the development of a simple, sensitive and accurate spectrophotometric method for the analysis of RPZ and OPZ in pure form as well as in pharmaceutical formulations. The described method is superior to the previously reported spectrophotometric methods in terms of the simplicity and sensitivity. The sample recoveries from all formulations were in good agreement with their respective label claims. The method provided excellent selectivity and linearity with a limit of detection of 0.181 and 0.187 μg mL⁻¹ for RPZ and OPZ, respectively. The proposed method has comparable
analytical performance and devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Therefore, this method can be recommended for the routine analysis of RPZ and OPZ in quality control laboratories.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mori M, Takata H, Fujisaki H. The potency of substituted benzimidazoles such as E3810, omeprazole, Ro 18-5364 to inhibit gastric H+, K+-ATPase is correlated with the rate of acid-activation of the inhibitor. Biochem. Pharmacol. 1990;9(4):661-667.
2. Martindale the Extra Pharmacopoeia, Royal Pharmaceutical Society, London, 2005;34th ed.:1283.
3. Antonio C, Morry M, Yogeshwar D, Carlos P, Robert N, Jay B. Rabeprazole for the prevention of pathologic and symptomatic relapse of erosive or ulcerative gastro esophageal reflux disease. Am. J. Gastroenterol. 2000;95(11):3081-3088.
4. Sluggett GW, Stong JD, Adams JH, Zhao Z. Omeprazole determination using HPLC with coulometric detection. J. Pharm. Biomed. Anal. 2001;25(3-4):357-361.
5. Garcia CV, Paim CS, Steppe M. New liquid chromatographic method for determination of rabeprazole sodium in coated tablets., J. AOAC Int. 2004;87(4):842-846.
6. Mehta DR, Mehta RS, Bhatt KK, Shankar MB. RP-HPLC method for estimation of rabeprazole sodium in bulk and in tablet dosage form, Indian Drugs. 2005;42(1):39-42.
7. El-Gindy A, El-Yazby F, Maher MM. Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products, J. Pharm. Biomed. Anal. 2003;31(2):229-242.
8. Ruiz M, Sierra J, Lopez-Viota M, Gallardo V. Spectrophotometric and chromatographic determination of omeprazole in pharmaceutical formulations. Pharm. Dev. Technol. 2009;14(5):516-23.
9. Patel BH, Patel MM, Patel JR, Suhagia BN. HPLC analysis for simultaneous determination of rabeprazole and domperidone in pharmaceutical formulation. J. Liq. Chromatogr. Relat. Technol. 2007;30(3):439-445.
10. Sabnis SS, Dnvandev DN, Jadhav VY, Gandhi SV. Column reversed-phase high-performance liquid chromatographic method for simultaneous determination of rabeprazole sodium and domperidone in combined tablet dosage form. J. AOAC Int. 2008;91(2):344-348.
11. Patel BH, Suhagia BN, Patel MM, Patel JR. High-performance liquid chromatography and thin-layer chromatography for the simultaneous quantitation of rabeprazole and mosapride in pharmaceutical products, J. Chromatogr. Sci. 2008;46(1):10-14.
12. Gandhi SV, Khan SI, Jadhav RT, Jadhav SS, Jadhav GA. High-performance thin-layer chromatographic determination of rabeprazole sodium and domperidone in combined dosage form. J. AOAC Int. 2009;92(4):1064-1067.
13. Patel BH, Suhagia BH, Patel MM, Patel JR. HPTLC determination of rabeprazole and domperidone in capsules and its validation. J. Chromatogr. Sci. 2008;46(4):304-307.
14. Tivesten A, Folestad S, Schonbacher V, Svensson K. Nonaqueous capillary electrophoresis for the analysis of labile pharmaceutical compound. Chromatographia. 1999;49(1):S7-S11.
15. Garcia CV, Sippel J, Sfair LL, Garcia SS, Jablonski A, Steppe M, et al. Validation of a capillary electrophoresis method for analysis of rabeprazole sodium in a pharmaceutical dosage form. J. AOAC Int. 2005;88(4):1081-1085.
16. Nevado JJB, Penalvo GC, Dodrardo RMR. Method development and validation for the separation and determination of omeprazole enantiomers in pharmaceutical preparations by capillary electrophoresis. Anal. Chim. Acta. 2005;533(2):127-133.
17. Radi A, Abd El-Ghany N, Wahdan T. Voltammetric behaviour of rabeprazole at a glassy carbon electrode and its determination in tablet dosage form. IL Farmaco. 2004;59(7):515-518.
18. Pinzauti S, Gratteri P, Furlanetto S, Mura P, Dreassi E, Phan-Tan-Luu R. Experimental design in the development of voltammetric method for the assay of omeprazole. J. Pharm. Biomed. Anal. 1996;14(8-10):881-889.
19. El-Enany N, Belal F, Rizk M. The alternating current polarographic behavior and determination of lansoprazole and omeprazole in dosage forms and biological fluids. J. Biochem. Biophys. Meth. 2008;70(6):889-896.
20. Rahman N, Bano Z, Azmi SN. Quantitative analysis of rabeprazole sodium in commercial dosage forms by spectrophotometry. Chem. Pharm. Bull. 2008;56(7):995-1001.
21. Patel P, Desai H, Patel R, Patel N. Spectrophotometric method for estimation of rabeprazole. Indian J. Pharm. Sci. 2007;69(2):318-320.
22. Syed A, Syeda A. Spectrophotometric determination of certain benzimidazole proton pump inhibitors. Indian J. Pharm. Sci. 2008;70(4):507-510.
23. Gupta P, Umamaheshwari RB, Rusia P, Dangi YS, Jain NK. Simultaneous estimation of amoxicillin trihydrate and rabeprazole sodium. Indian J. Pharm. Sci. 2005;67(3):380-382.
24. Sabnis SS, Dhavele ND, Jadhav VY, Gandhi SV. Spectrophotometric simultaneous determination of rabeprazole sodium and itopride hydrochloride in capsule dosage form. Spectrochim. Acta Part A. 2008;69(3):849-852.
25. Pattanayak P, Sharma R, Chaturvedi SC. Simultaneous spectrophotometric estimation of rabeprazole sodium and itopride HCl. Anal. Lett. 2007;40(12):2288-2294.
26. Heralgi R, Simpi C, Kalyane N, Karaji S. Simultaneous spectrophotometric estimation of rabeprazole sodium and itopride hydrochloride in capsule formulations. Asian J. Pharm. 2008;2(3):148-149.
27. Garcia CV, Sippel J, Steppe M, Schapoval EES. Development and validation of derivative spectrophotometric method for determination of rabeprazole sodium in pharmaceutical formulation. Anal. Lett. 2006;39(1-3):341-348.
28. Saudagar RB, Saraf S. First order derivative simultaneous equation and area under curve method for estimation of domeridone maleate and rabeprazole sodium in tablet dosage form. Indian Drugs. 2006;43(5):388-392.
29. Lotfy HM, Hagazy MA. Comparative study of novel spectrophotometric methods manipulating ratio spectra: An application on pharmaceutical ternary mixture of omeprazole, tinidazole and clarithromycin. Spectrochim. Acta Part A. 2012;96:259-270.
30. Zhang Y, Chen X, Gu Q, Zhong D. Quantification of rabeprazole in human plasma by liquid chromatography–tandem mass spectrometry. Anal. Chim. Acta. 2004;523(2):171-175.
31. Ramakrishna NV, Vishwottam KN, Wishu S, Koteshwara M, Kumar S. High-performance liquid chromatography method for the quantification of rabeprazole in human plasma using solid-phase extraction. J. Chromatogr. B. 2005;816(1-2):209-214.
32. Huang J, Xu Y, Gao S, Rui L, Guo Q. Development of a liquid chromatography/tandem mass spectrometry assay for the quantification of rabeprazole in human plasma. Rapid Commun. Mass Spectro. 2005;19(16):2321-2324.

33. Hishinuma T, Suzuki K, Yamaguchi H, Yamagisho H, Koike T, Ohara S, et al. Simple quantification of lansoprazole and rabeprazole concentrations in human serum by liquid chromatography/tandem mass spectrometry. J. Chromatogr. B. 2008;870(1):38-45.

34. Nozal MJ, Tor´Ibio L, Bernal J, Alonso C, Jimenez J. Chiral separation of omeprazole and several related benzimidazoles using supercritical fluid chromatography. J. Sep. Sci. 2004;27(11):1023-1029.

35. Rao RN, Raju AN, Nagaraju D. Enantiospecific resolution of rabeprazole by liquid chromatography on amylose-derived chiral stationary phase using photodiode array and polarimetric detectors in series. Talanta. 2006;70(4):805-810.

36. United States Pharmacopoeia, United States Pharmacopeial Convention, Rockville, 2011;34th ed.

37. British Pharmacopoeia, Her Majesty's Stationery Office Ltd., London. 2009;4536.

38. Darwish IA, Abdine HH, Amer SM, Al-Rayes LI. Spectrophotometric study for the reaction between fluvoxamine and 1,2-naphthoquinone-4-sulphonate: kinetic, mechanism and use for determination of fluvoxamine in its dosage forms. Spectrochim. Acta Part A. 2009;72(4):897-902.

39. Wang HY, Xu LX, Xiao Y, Han J. Spectrophotometric determination of dapsone in pharmaceutical products using sodium 1,2-naphthoquinone-4-sulfonic as the chromogenic reagent. Spectrochim. Acta Part A. 2004;60(12):2933-2939.

40. ROSE J, Advanced physico-chemical experiments. Pittman, London. 1964;67.

41. Harvey AE, Manning DL. Spectrophotometric methods of establishing empirical formulas of colored complexes in solution. J. Am. Chem. Soc. 1950;72(10):4488-4493.

42. Morelli B. Determination of iron(III) and copper(II) by zeroth, first and second derivative spectrophotometry with 2-thiobarbituric acid (4,6-dihydroxy-2-mercaptopurimidine) as reagent. Analyst. 1983;108(1288):870-879.

43. Nalimov VV. The application of Mathematical Statistics to Chemical Analysis. Pergamon Press Oxford. 1963;189.

44. Miller JC, Miller JN. Statistics in analytical chemistry. Ellis Horwood, Chichester, London. 1993;3rd ed.:119.