Spectrophotometric Determination of Omeprazole Sodium and Ranitidine Hydrochloride Using 2, 4-dinitrofluorobenzene and 4-chloro-7-nitrobenzen-2-oxa-1, 3-diazole Accompanied with a Kinetic Study

Mohamed M. Baraka, Mohamed E. El-Sadek, Lobna M. Abdel-Aziz and Samar S. El-bermawi*

Medicinal Chemistry Department, Faculty of Pharmacy, Zagazig University, P.C. 44519, Zagazig, Egypt

Abstract:
Two accurate, simple and sensitive spectrophotometric methods were developed for determination of omeprazole sodium and ranitidine hydrochloride in bulk and pharmaceutical dosage forms. The first method was based on the reaction of these drugs with 2, 4-dinitrofluorobenzene producing yellow colored products measured at $\lambda_{\text{max}}$ 470 nm and 420 nm for omeprazole sodium and ranitidine HCl, respectively. Beer’s law was obeyed in the concentration range from (5-40 µg.ml$^{-1}$) for omeprazole sodium and (30-180 µg.ml$^{-1}$) for ranitidine HCl with molar absorptivity 9.202 x 10$^3$ Lmol$^{-1}$cm$^{-1}$ and 1.778 x 10$^3$ Lmol$^{-1}$cm$^{-1}$, respectively. The second method was based on the reaction of omeprazole sodium and ranitidine HCl with 4-Chloro-7-nitrobenzen-2-oxa-1, 3-diazole producing yellow colored adducts measured at $\lambda_{\text{max}}$ 481 nm and 468 nm for omeprazole sodium and ranitidine HCl, respectively. This method was accompanied with a kinetic study for ranitidine HCl. The absorbance-concentration plots were rectilinear over concentration range (2-12 µg.ml$^{-1}$) and (0.025-0.15 µg.ml$^{-1}$) for omeprazole sodium and ranitidine HCl with molar absorptivity 4.062 x 10$^4$ Lmol$^{-1}$cm$^{-1}$ and 2.802 x 10$^6$ Lmol$^{-1}$cm$^{-1}$, respectively. These methods hold their accuracy and precision well when applied to the determination of omeprazole sodium and ranitidine HCl in their dosage forms.

Keywords: omeprazole sodium; ranitidine hydrochloride; 2, 4-dinitrofluorobenzene; 4-Chloro-7-nitrobenzen-2-oxa-1, 3-diazole

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Competing Interests: The authors have declared that no competing interests exist.

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*Correspondence to: Samar S. El-bermawi, Medicinal Chemistry Department, Faculty of Pharmacy, Zagazig University, P.C. 44519, Zagazig, Egypt. Email: samar_elbermawi@yahoo.com
Introduction

Omeprazole sodium is chemically known as sodium salt of 5-methoxy-2-[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl] benzimidazole.

Omeprazole is officially listed in B.P.2011 [1] and U.S.P.XXXII [2]. It is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-esophageal reflux disease and the Zollinger-Ellison syndrome [3].

A survey of the literature revealed that omeprazole has been estimated in pharmaceuticals by UV-spectrophotometry [4-6], spectrofluorimetry [7], flow injection method [8], HPLC [9-11], HPTLC [12, 13], capillary electrophoresis [14] and electrochemical methods [15, 16].


Ranitidine HCl is a histamine H2-receptor antagonist and inhibits the actions of histamine mediated by H2-receptors such as gastric acid secretion and pepsin output. It is used where inhibition of gastric acid secretion may be beneficial as in peptic ulcer disease including stress ulceration, gastroesophageal reflux disease and selected cases of persistent dyspepsia [3].

Various analytical methods have been reported for the assay of ranitidine HCl in its pure form as well as in pharmaceutical formulations. These methods include spectrophotometric, [17-19] potentiometric methods [20, 21], and HPLC methods [22-24].

This paper reports simple, sensitive, economical and accurate spectrophotometric methods for the analysis of omeprazole sodium and ranitidine hydrochloride in their pure and dosage forms. The results of the analysis were validated by statistical analysis and recovery studies. Common additives used as excipients in...
pharmaceutical dosage forms do not interfere in the determination of the studied drugs.

**Material and methods**

Apparatus:
- Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells connected to windows compatible computer using UV Win 5 Software v5.0.5.
- Digital pH-meter (Cosort P400) ® for pH adjustment.
- Thermostatically controlled (Wisebath) ® water bath.

Materials and reagents:
All solvents and reagents were of analytical grade and distilled water was used throughout the work.

Materials:
- Omeprazole sodium (Hikma Pharmaceutical Co., 6th of October City, Egypt).
- Ranitidine hydrochloride (Sigma Pharmaceutical Industries, Quesna City, Egypt).

Pharmaceutical preparations:
The following pharmaceutical preparations were analyzed:
- Pepzol® capsules (Hikma Pharmaceutical Co., 6th of October City, Egypt) labeled to contain 20 mg omeprazole per capsule.
- Aciloc® tablets (Sigma Pharmaceutical Industries, Quesna City, Egypt) labeled to contain 300 mg ranitidine HCl per tablet.
- Zantac® ampoules (Glaxo Smithkline, Egypt) labeled to contain 50 mg ranitidine HCl per ampoule.

Reagents:
- 2, 4- Dinitrofluorobenzene (DNFB; Sanger’s reagent) (Acros, Newjersey, USA).
- 4- Chloro-7-nitrobenzen-2-oxa-1, 3-diazole (NBD-Cl) (Acros, Newjersey, USA).
- Sodium hydroxide (Sd fine-chem limited, industrial estate, Mumbai, India).
- Boric acid, hydrochloric acid and methanol (EL-Nasr Pharm.Chem.Co., Egypt).

Preparation of materials and reagents:

**Working solutions:**

**Pure drugs:**
- Method I: standard solutions of omeprazole sodium and ranitidine hydrochloride (100 µg/ml and 600 µg/ml) were prepared by dissolving 10 mg and 60 mg of the pure drug respectively in 100 ml distilled water in a volumetric flask.
- Method II: standard solutions of omeprazole sodium and ranitidine hydrochloride (40 µg/ml and 1 µg/ml) were prepared by dissolving 4 mg and 0.1 mg of the pure drug, respectively in 100 ml distilled water in a volumetric flask.

**2, 4-Dinitrofluorobenzene (DNFB; Sanger’s reagent):**
- Fresh solution of 0.3%v/v DNFB in methanol was prepared daily.

**4-Chloro-7-nitrobenzen-2-oxa-1, 3-diazole (NBD-Cl):**
- Fresh solution of 0.4%w/v NBD-Cl in methanol was prepared daily.

**Borate buffer solutions:**
- Borate buffer solutions (0.2 M) were prepared by mixing appropriate volumes of 0.2 M boric acid and 0.2 M NaOH (2) and adjusting the pH to 9.8 and 10.8 using the pH-meter.

**General Procedures:**
- Authentic drugs:
  - Method I
    - Accurately measured aliquots of standard solutions containing (50-400 µg/ml) and (300-1800 µg/ml) of
Omeprazole sodium and ranitidine hydrochloride respectively were transferred into a series of 10-ml volumetric flasks, to each flask, 1.5 ml of borate buffer (pH = 10.5) and 0.4 ml and 1.5 ml of 0.3% v/v DNFB were added for omeprazole sodium and ranitidine HCl respectively and mixed well. The solutions were heated at 70°C and 80°C for 15 and 20 minutes in thermostatically controlled water bath for omeprazole sodium and ranitidine HCl respectively. The solutions were cooled and acidified with 1 ml of 0.1 M HCl. The reaction products were diluted to 10 ml with methanol. The absorbances were measured at λ max 470 nm and 420 nm for omeprazole sodium and ranitidine HCl against the blank.

Method II

Accurately measured aliquots of standard solutions containing (20-120 µg/ml), (0.25-1.5 µg/ml) of omeprazole sodium and ranitidine hydrochloride, respectively were transferred into a series of 10-ml volumetric flasks.

To each flask, 1 ml of borate buffer (pH = 10.8) and 1 ml of NBD-Cl solution were added in case of omeprazole sodium and 0.8 ml of borate buffer (pH = 9.8) and 1 ml of NBD-Cl solution were added in case of ranitidine HCl and mixed well.

The solutions were heated for 15, 25 minutes in thermostatically controlled water bath (80, 60°C) for omeprazole sodium and ranitidine HCl respectively.

The solutions were cooled and acidified with 1 ml of 0.1 M HCl. The reaction products were diluted to 10 ml with methanol.

The absorbances were measured at λ max 481 nm and 468 nm for omeprazole sodium and ranitidine HCl against the blank.

Pharmaceutical preparations:

Capsules:
The contents of twenty capsules of Pepzol were emptied and pulverized.

An accurately weighed amount equivalent to concentration of omeprazole taken in method I and II were extracted by shaking with 5 ml 0.01N NaOH three times, the filtrates were collected and transferred to 100 ml volumetric flask, completed to the mark with distilled water. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

Tablets

Twenty tablets of Aciloc were weighed and ground into a fine powder.

An accurately weighed amount of the powder equivalent to the concentration of ranitidine HCl in method I and II were extracted with distilled water three times, the filtrates were collected and transferred to 100 ml volumetric flask and completed to the mark with distilled water. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed method applying standard addition techniques.

Ampoules:

Ten ampoules of Zantac were evacuated in 250 ml volumetric flask; the volume was adjusted to 250 ml using distilled water. Aliquots from this solution equivalent to those in authentic samples were used for the application of the proposed method applying standard addition techniques for both methods.

Results and Discussion

2, 4-Dinitrofluorobenzene (DNFB; Sanger’s reagent) is an active aryl halide reacts with primary and secondary amines, phenols, thiols and imidazoles forming stable condensation coloured products [25].

DNFB has been utilized as a chromogen and a fluorogen for the spectrophotometric [26-29] and spectrofluorometric [30, 31] determination of many compounds of pharmaceutical interest.
Also some drugs have been derivatized with DNFB before estimation with HPLC [30, 32].

4-chloro-7-nitrobenzo-1, 3-diazone (NBD-Cl), is an electroactive halide reagent. It is well known to react with primary, secondary amines and thiol groups forming stable condensation coloured products, so it has been used as chromogenic [33-36] or fluorogenic [37, 38] reagent in pharmaceutical analysis. It has also been used in charge transfer reactions due to its electrophilic properties, where it acts as π-acceptor as in determination of some β-blockers [39], skeletal muscle relaxants and antihistaminic drugs [40].

This paper shows the possibility of the reaction of DNFB and NBD-Cl with omeprazole sodium and ranitidine HCl in alkaline media through nucleophilic substitution reactions. These drugs contain secondary amino group which can react with DNFB and NBD-Cl, producing yellow coloured products, shown in reaction schemes. This study is accompanied with a kinetic study for the reaction of ranitidine HCl with NBD-Cl. The suggested mechanism is explained below.

![Reaction scheme between omeprazole sodium and DNFB](image)

**Scheme (1)** Reaction scheme between omeprazole sodium and DNFB
Scheme (2) Reaction scheme between ranitidine HCl and DNFB

Scheme (3) Reaction scheme between omeprazole sodium and NBD-Cl
Study of the experimental parameters:

The different experimental parameters affecting the development of the reaction products were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors include pH, type of buffer, reagent concentration, temperature, heating time and effect of diluting solvent.

i- Effect of pH and volume of buffer

Different buffers (borate, phosphate, hexamine) were studied. Borate buffer was found to be the most suitable buffer for both methods, Fig. (3 and 4). For method I; 1.5 ml of borate buffer (pH=10.5) for omeprazole sodium and ranitidine HCl gave best results. While for method II; the best results were obtained on using 1 ml of borate buffer (pH=10.8) in case of omeprazole sodium and 0.8 ml of borate buffer (pH= 9.8) in case of ranitidine HCl. Fig. (5 and 6).

ii- Effect of the reagent concentration.

The concentrations of the reagents were investigated. For method I; 0.4 ml and 1.5ml of 0.3%v/v DNFB solution for omeprazole sodium and ranitidine HCl were optimum reagent concentrations. While for method II; 1ml of 0.4%w/v NBD-Cl solution for omeprazole sodium and ranitidine HCl were sufficient for production of maximum and reproducible color intensity. Fig. (7 and 8).

iii- Effects of temperature and heating time
It was found that the reaction proceeds very slowly at room temperature. A gradual increase in the temperature produced a significant increase in the absorbance. For method I; the reaction proceeded at 70°C and 80°C for 15 and 20 minutes for omeprazole sodium and ranitidine HCl, respectively. For method II; the reaction was carried out at 80°C and 60°C for 15 and 25 minutes for omeprazole sodium and ranitidine HCl respectively. Fig. (9, 10, 11 and 12).

iv- Effect of HCl addition

Acidification of the reaction mixture prior to measurement of the absorbance value remarkably decreased the background absorbance. In method I; acidification of the medium with 1 ml of 0.1M HCl transfers yellow 2, 4-dinitrophenolate into colorless 2, 4-dinitrophenol[41]. While in method II; the absorbance of the hydrolysis product of NBD-Cl, namely, 4-hydroxy-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-OH) is quenched by decreasing the pH of the reaction medium using 1 ml of 0.1M HCl [42]. Fig (13 and 14).

v- Effect of diluting solvent

Different organic solvents were tested as methanol, acetone, acetonitrile, dimethylformamide and isopropanol, methanol gives reasonable absorption intensity with maximum product stability for method I and II.

Stoichiometry of the reaction:

The molar ratio of the reagent and both drugs in the reaction mixture was studied according to Job’s method of continuous variation [43]. The molar ratio was found to be 1:1 (drug: reagent) in both methods.

Validation of the proposed method

The validity of the proposed methods was tested according to ICH recommendations [44].

Linearity and Quantification

Method I

A linear relationship was obtained for the absorbance of DNFB with the two cited drugs in the concentration ranges of (5-40 µg.ml-1) and (30-180 µg.ml-1) for omeprazole sodium and ranitidine HCl respectively.

Method II

A linear relationship was obtained for the absorbance of NBD-Cl with the two cited drugs in the concentration ranges of (2-12 µg.ml-1) and (0.025-0.15 µg.ml-1) for omeprazole sodium and ranitidine HCl respectively.

Under the optimized conditions, the optical and statistical parameters for the proposed methods are summarized in table (1). The molar absorptivity, Sandell’s sensitivity, correlation coefficients, slopes and intercepts were listed.

The good linearity of the calibration graph and the negligible scatter of the experimental points were clearly evident from the value of the correlation coefficient and variance.

The proposed methods were successfully applied for the determination of pure drugs. Performance of the proposed methods was assessed by comparing the calculated t and F values with the reference
methods [45, 46]. The results showed that the t and F values were less than the tabulated ones indicating that there was no significant difference between the proposed and reference methods. Table (2)

Dosage forms containing omeprazole and ranitidine HCl were analyzed by the proposed methods applying the standard addition technique. The obtained results compared with the reference method and statistical analysis of the results showed that there is no interference from the common additive and excipients, indicating a high selectivity for determining the studied drugs in their dosage forms. Table (3)

Sensitivity

The limit of detection (LOD) for the two spectrophotometric methods was calculating using the following equation:

$$\text{LOD} = 3.3S/K$$

The limit of quantification, LOQ is defined as:

$$\text{LOQ} = 10S/K$$

Where S is the standard deviation of the three replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph.

According to these equations, the limits of detection and the limits of quantification were calculated and are listed in Table (1).

Accuracy

The accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique. The results are shown in table (3). No interference from the excipients was observed.

Intraday precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug. In the method I; the SD values were 0.109 and 0.118 for omeprazole sodium and ranitidine HCl, respectively. For method II; the SD values were 0.099 and 0.115 for omeprazole sodium and ranitidine HCl, respectively. The small values of SD revealed the precision of the method.

For interday reproducibility on a day-to-day basis, a series was run, in which the standard drug solutions were analyzed each for five days. For method I; the interday SD values were 0.174 and 0.221 for omeprazole sodium and ranitidine HCl respectively. For method II; the interday SD values were 0.193 and 0.188 for omeprazole sodium and ranitidine HCl respectively.

The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable.

Robustness

Robustness of the methods was examined by small changes in the method variables such as change in pH (± 0.2), change in the volume of the reagent (± 0.1 ml) and change in temperature (± 5°C).

The minor changes that may take place during the experiment didn’t affect the absorbance of the reaction products.

Kinetic study of the reaction between ranitidine HCl and NBD-Cl:
The rate of the reaction was found to be concentration dependent. The rate of the reaction was followed with various concentrations of ranitidine HCl in the range of 0.025-0.15 µg/ml. The graph shown in (fig.15) indicates that the reaction rate of ranitidine HCl obeys the following equation:

\[ \text{Rate} = k' \ [\text{drug}]^n \]  

(1)

Where \( k' \) is the pseudo-order rate constant and \( n \) is the order of the reaction.

The rate of the reaction may be estimated by the variable-time method (47).

In this method the reaction rate was followed by measuring the change of absorbance at different time intervals. Taking logarithms of rates and concentration (Table 4) equation (1) is transformed into:

\[ \log (\text{rate}) = \log \frac{\Delta A}{\Delta t} = \log k' + n \log [\text{drug}] \]  

(2)

Where \( A \) is the absorbance and \( t \) is the time in seconds.

Regression of \( \log \) (rate) versus \( \log \) (drug) gave the following regression equations:

\[ \log (\text{rate}) = 3.159 + 0.986 \log [\text{drug}] \]

Where \( r = 0.986 \) and \( k' = 1.442 \times 10^3 \text{s}^{-1} \)

Hence the reaction is first order (\( n \approx 1 \)) with respect to drug concentration.

Evaluation of the kinetic methods:

Several experiments were carried out to obtain the drug concentration from the rate data according to the corresponding equation (1). Rate constant, fixed absorbance and fixed time methods were tried and the most suitable analytical method was selected taking in consideration applicability, sensitivity and correlation coefficient.

Rate constant method:

Graph of \( \log \) (absorbance) versus time in seconds over the concentration range 7.12x10^{-8} – 4.27x10^{-7} M was plotted and the pseudo-first order constant (\( k' \)) corresponding to different drug concentrations were calculated from slopes multiplied by -2.303 and presented in (Table 5, Fig.16).

\[ k' = -1x10^{-3} – 9.341x10^2 \text{C} \]

\[ r^2 = 0.887 \]

Fixed absorbance method:

Reaction rates were recorded for different concentrations of ranitidine HCl (7.12x10^{-8} – 4.27x10^{-7} M). A preselected value of absorbance 0.36 was fixed and time was measured in seconds. The reciprocal of time (1/t) versus the different drug concentrations was plotted (Table 6, fig. 17).

\[ 1/t = 3.6 \times 10^{-4} + 4.28 \times 10^3 \text{C} \]

\[ r^2 = 0.9963 \]

Fixed time method:

Reaction rates were determined for different concentrations of ranitidine HCl.

Calibration graph of absorbance versus initial concentration of ranitidine HCl was established at fixed time of 5, 10, 15, 20, and 25 and 30 minutes with the calibration equations (Table 7). It was clear that the slope and the correlation coefficient increase with time. The best correlation coefficient for ranitidine HCl was obtained for a fixed time of 25 minutes; therefore this fixed time was used for its measurement.
Table 1 Analytical parameters and spectral data for spectrophotometric determination of omeprazole sodium and ranitidine HCl through method I and II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I (DNFB)</th>
<th>Method II (NBD-Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Omeprazole sodium</td>
<td>Ranitidine HCl</td>
</tr>
<tr>
<td>λmax, nm</td>
<td>470 nm</td>
<td>420 nm</td>
</tr>
<tr>
<td>Borate buffer ;pH</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Borate buffer volume (ml)</td>
<td>1.5 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>Reagent Conc.</td>
<td>0.3%v/v</td>
<td>0.3%v/v</td>
</tr>
<tr>
<td>Reagent volume (ml)</td>
<td>0.4 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>70°C</td>
<td>80°C</td>
</tr>
<tr>
<td>Reaction time (min.)</td>
<td>15 min.</td>
<td>20 min.</td>
</tr>
<tr>
<td>Diluting solvent</td>
<td>Methanol</td>
<td>Methanol</td>
</tr>
<tr>
<td>Beer’s law limits (µg.ml-1)</td>
<td>5-40 µg/ml</td>
<td>30-180 µg/ml</td>
</tr>
<tr>
<td>Regression equation*</td>
<td>Slope (b)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Intercept (a)</td>
<td>0.074</td>
</tr>
<tr>
<td>Variance</td>
<td>0.488</td>
<td>0.236</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Molar absorptivity**</td>
<td>9.202 x 103</td>
<td>1.778 x 103</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg.cm-2)</td>
<td>2.02 x 10-2</td>
<td>1.04 x 10-1</td>
</tr>
<tr>
<td>Limit of detection; LOD(µg.ml-1)</td>
<td>1.48</td>
<td>7.44</td>
</tr>
<tr>
<td>Limit of quantification; LOQ(µg.ml-1)</td>
<td>4.51</td>
<td>22.55</td>
</tr>
</tbody>
</table>

*A = a + bC where A is absorbance, C is the concentration of the drug in µg.ml-1; **Calculated in the basis of molecular weight of the drug.
Table 2 Statistical data for determination of omeprazole sodium and ranitidine HCl using method I and method II compared with the reference method.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Omeprazole sodium</th>
<th>Ranitidine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference method</td>
<td>Method I</td>
</tr>
<tr>
<td>Mean recovery*± SD</td>
<td>100±0.700</td>
<td>99.65±0.418</td>
</tr>
<tr>
<td>RSD</td>
<td>0.69</td>
<td>0.419</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Variance</td>
<td>0.53</td>
<td>0.175</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.313</td>
<td>0.187</td>
</tr>
<tr>
<td>t-test**</td>
<td>0.961</td>
<td>1.827</td>
</tr>
<tr>
<td>F-test**</td>
<td>2.80</td>
<td>1.988</td>
</tr>
</tbody>
</table>

* Average of three experiments; **Theoretical t and F values are 2.306 and 5.05, respectively at p=0.05.
Table 3 Statistical data for determination of pharmaceutical preparation of omeprazole sodium and ranitidine HCl through method I and method II compared with the reference method.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Omeprazole sodium</th>
<th>Ranitidine HCl</th>
<th>Ranitidine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pepzol® capsules</td>
<td>Aciloc® tablets</td>
<td>Zantac® ampoules</td>
</tr>
<tr>
<td>Mean recovery</td>
<td>100±0.70</td>
<td>100.79±0.65</td>
<td>100.85±0.722</td>
</tr>
<tr>
<td>SD</td>
<td>0.912</td>
<td>0.65</td>
<td>0.596</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Variance</td>
<td>0.53</td>
<td>0.342</td>
<td>0.310</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.313</td>
<td>0.409</td>
<td>0.320</td>
</tr>
<tr>
<td>t-test**</td>
<td>1.539</td>
<td>1.99</td>
<td>0.860</td>
</tr>
<tr>
<td>F-test**</td>
<td>1.145</td>
<td>1.16</td>
<td>1.467</td>
</tr>
</tbody>
</table>

* Average of three experiments; **Theoretical t and F values are 2.306 and 5.05, respectively at p=0.05.

Table 4 Logarithms of rates for different concentrations of ranitidine HCl

<table>
<thead>
<tr>
<th>Log ΔA/Δt</th>
<th>Log [conc.] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.883</td>
<td>-7.14</td>
</tr>
<tr>
<td>-3.603</td>
<td>-6.84</td>
</tr>
<tr>
<td>-3.407</td>
<td>-6.67</td>
</tr>
<tr>
<td>-3.301</td>
<td>-6.54</td>
</tr>
<tr>
<td>-3.191</td>
<td>-6.44</td>
</tr>
<tr>
<td>-3.123</td>
<td>-6.37</td>
</tr>
</tbody>
</table>
Table 5 Values of $k'$ calculated from slopes of log $A$ versus time for ranitidine HCl

<table>
<thead>
<tr>
<th>$K'$ (S$^{-1}$)</th>
<th>[conc.] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.98 x 10^{-3}</td>
<td>7.12 x 10^{-8}</td>
</tr>
<tr>
<td>-1.1 x 10^{-3}</td>
<td>1.42 x 10^{-7}</td>
</tr>
<tr>
<td>-1.25 x 10^{-3}</td>
<td>2.13 x 10^{-7}</td>
</tr>
<tr>
<td>-1.25 x 10^{-3}</td>
<td>2.85 x 10^{-7}</td>
</tr>
<tr>
<td>-1.37 x 10^{-3}</td>
<td>3.56 x 10^{-7}</td>
</tr>
<tr>
<td>-1.38 x 10^{-3}</td>
<td>4.27 x 10^{-7}</td>
</tr>
</tbody>
</table>

Table 6 Values of reciprocal of time taken at fixed absorbance for different rates of variable concentrations of ranitidine HCl

<table>
<thead>
<tr>
<th>$1/t$ (s$^{-1}$)</th>
<th>[conc.] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.95 x 10^{-4}</td>
<td>1.43 x 10^{-7}</td>
</tr>
<tr>
<td>1.25 x 10^{-3}</td>
<td>2.14 x 10^{-7}</td>
</tr>
<tr>
<td>1.58 x 10^{-3}</td>
<td>2.85 x 10^{-7}</td>
</tr>
<tr>
<td>1.85 x 10^{-3}</td>
<td>3.56 x 10^{-7}</td>
</tr>
<tr>
<td>2.22 x 10^{-3}</td>
<td>4.28 x 10^{-7}</td>
</tr>
</tbody>
</table>

$1/t = 3.6 x 10^{-4} + 4.28 x 10^{-3}$ C

$r^2 = 0.9963$

Table 7 Regression equations at different fixed time for ranitidine HCl in concentration range 7.12 x 10^{-8} – 4.27 x 10^{-7} M.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Regression equations</th>
<th>Correlation coefficient ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.0634 + 0.7897 C</td>
<td>0.9832</td>
</tr>
<tr>
<td>10</td>
<td>0.0374 + 3.144 C</td>
<td>0.9888</td>
</tr>
<tr>
<td>15</td>
<td>0.1036 + 3.850 C</td>
<td>0.9926</td>
</tr>
<tr>
<td>20</td>
<td>0.1128 + 5.032 C</td>
<td>0.9882</td>
</tr>
<tr>
<td>25</td>
<td>0.0686 + 6.828 C</td>
<td>0.9996</td>
</tr>
<tr>
<td>30</td>
<td>0.005 + 6.811 C</td>
<td>0.9833</td>
</tr>
</tbody>
</table>
Fig. 1 Absorption spectra of (method I) for the reaction between DNFB and 10 µg.ml-1 omeprazole sodium at λ_{max} 470 nm and 80 µg.ml-1 ranitidine HCl at λ_{max} 420 nm.

Fig. 2 Absorption spectra of (method II) for the reaction between NBD-Cl and 6µg.ml-1 omeprazole sodium at λ_{max} 481 nm and 0.05 µg.ml-1 ranitidine HCl at λ_{max} 468 nm.
Fig. 3 Effect of buffer pH on the reaction of DNFB with 10 µg.ml\(^{-1}\) omeprazole sodium and 60 µg.ml\(^{-1}\) ranitidine HCl.

Fig. 4 Effect of buffer pH on the reaction of NBD-Cl with 4 µg.ml\(^{-1}\) omeprazole sodium and 1µg.ml\(^{-1}\) ranitidine HCl.

Fig. 5 Effect of buffer volume on the reaction of DNFB with 10 µg.ml\(^{-1}\) omeprazole sodium and 60 µg.ml\(^{-1}\) ranitidine HCl.
**Fig. 6** Effect of buffer volume on the reaction of NBD-Cl with 4 µg.ml⁻¹ omeprazole sodium and 1 µg.ml⁻¹ ranitidine HCl.

**Fig. 7** Effect of DNFB volume (ml) on the reaction of DNFB with 10 µg.ml⁻¹ omeprazole sodium and 60 µg.ml⁻¹ ranitidine HCl.

**Fig. 8** Effect of NBD-Cl volume (ml) on the reaction of NBD-Cl with 4 µg.ml⁻¹ omeprazole sodium and 1 µg.ml⁻¹ ranitidine HCl.
Fig. 9 Effect of temperature (°C) on the reaction of DNFB with 10 μg.ml⁻¹ omeprazole sodium and 60 μg.ml⁻¹ ranitidine HCl.

Fig. 10 Effect of temperature (°C) on the reaction of NBD-Cl with 4 μg.ml⁻¹ omeprazole sodium and 1 μg.ml⁻¹ ranitidine HCl.

Fig. 11 Effect of heating time (min.) on the reaction of DNFB with 10 μg.ml⁻¹ omeprazole sodium and 60 μg.ml⁻¹ ranitidine HCl.
**Fig. 12** Effect of heating time (min.) on the reaction of NBD-Cl with 4 µg.ml⁻¹ omeprazole sodium and 1 µg.ml⁻¹ ranitidine HCl.

**Fig. 13** Effect of volume of 0.1M HCl on the reaction of DNFB with 10 µg.ml⁻¹ omeprazole sodium and 60 µg.ml⁻¹ ranitidine HCl.

**Fig. 14** Effect of volume of 0.1M HCl on the reaction of NBD-Cl with 4 µg.ml⁻¹ omeprazole sodium and 1 µg.ml⁻¹ ranitidine HCl.
Fig. 15 Absorbance versus time graphs for the reaction between ranitidine HCl and NBD-Cl at different concentrations of ranitidine HCl.

1 = 7.12x10^-8 M
2 = 1.42x10^-7 M
3 = 2.13x10^-7 M
4 = 2.85x10^-7 M
5 = 3.56x10^-7 M
6 = 4.27x10^-7 M

Fig. 16 Values of k’(s-1) calculated from slopes of different log absorbance versus t graph multiplied by -2.303 for different concentrations of ranitidine HCl.
Conclusion

The proposed spectrophotometric methods provided simple, sensitive, specific and inexpensive analytical procedures for determination of the cited drugs either in pure forms or in their pharmaceutical formulations without interference from common excipients. The satisfactory sensitivity and reproducibility as well as the convenience and simplicity, make the two proposed methods suitable for routine analysis in quality control laboratories.

References

7. Shagaghi M, Manzoori JL, Jouyban A. Indirect spectrofluorimetric determination of omeprazole by its quenching effect on the fluorescence of Tb3+·1,10-phenanthroline complex in presence of bis (2-ethylhexyl) sulfosuccinate sodium in capsule formulations. DARU J. Pharm. Sci. 2008, 16:256-262
9. El-Sherif AZ, Mohamed OA, El-Bardicy GM, El-Tarras FM, Reversed-phase high performance liquid chromatographic method for the determination of lansoprazole, omeprazole and pantoprazole sodium sesquihydrate


38. Ulu ST. Highly sensitive determination and validation of gabapentin in pharmaceutical preparations by HPLC with 4-fluoro-7-nitrobenzofurazan derivatization and fluorescence detection. *Pharmazie.* 2011, 66:831-5


42. Armagan O. Spectrophotometric and spectrofluorometric determination of some drugs containing secondary amino group in bulk drug and dosage forms via derivatization with 7-Chloro-4-Nitrobenzofurazan. *Quim Nova.* 2011, 34:677-682


