Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 6, Issue 3, 2014

Research Article

SPECTROPHOTOMETRIC DETERMINATION OF OMEPRAZOLE VIA NITROSATION REACTION

MOHAMED M. BARAKA, MOHAMED E. ELSADEK, LOBNA M. ABDELAZIZ AND SAMAR S. ELBERMAWI*

Medicinal Chemistry Department, Faculty of Pharmacy, Zagazig University, P.C. 44519, Zagazig, Egypt.
Email: samar_elbermawi@yahoo.com

Received: 03 April 2014, Revised and Accepted: 24 May 2014

ABSTRACT

A simple, sensitive and reproducible spectrophotometric method is developed for determination of omeprazole in bulk and capsules. The method is based on the nitrosation reaction of the drug, producing yellow colored product measured at λ_{max} 390 nm. The absorbance is proportional to omeprazole concentration in the range 20-110 µg ml^{-1}. The validity of the proposed method was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The suggested method could be used for the determination of omeprazole in capsules. The procedures are rapid, simple and suitable for quality control application.

Keywords: Omeprazole, Spectrophotometry, Sodium nitrite, Nitrosation reaction.

INTRODUCTION

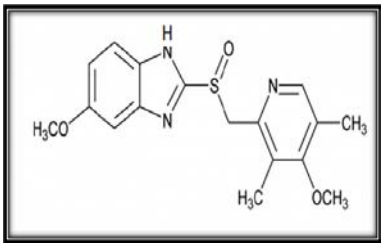


Fig. 1: Structure of omeprazole

Omeprazole is chemically known as 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 characterized as a gastric acid pump inhibitor because it blocks the final step of gastric acid production. It has a long duration of action and is very potent, allowing for once-daily administration. Despite its potency, it must be used in combination with antibiotics to be effective against *Helicobacter pylori* [3]. A survey of the literature revealed that omeprazole has been estimated in pharmaceuticals by UV-spectrophotometry [4-6], spectrofluorimetry [7], HPLC [8-10], HPTLC [11], capillary electrophoresis [12] and electrochemical methods [13 and 14]. This study reports simple, sensitive, economical and accurate spectrophotometric method for the quantitative estimation of omeprazole in its pure form and dosage form. The results of the analysis were validated by statistical analysis and recovery studies. Common additives used in pharmaceutical formulation do not interfere in the determination of the cited drug.

MATERIALS 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.

Materials

Omeprazole (Sigma Pharmaceutical Industries, Quesna City, Egypt).

Pharmaceutical preparation (capsules)

The following pharmaceutical capsules were analyzed:

Pepzol® capsules (Hikma Pharmaceutical Co., 6th of October City, Egypt) labeled to contain 20 mg omeprzole per capsule.

Reagents

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

Sodium hydroxide and nitric acid (Sd fine-chem limited, industrial estate, Mumbai, India) Sodium nitrite (EL-Nasr Pharm. Chem. Co., Egypt)

Preparation of standard drug solution

Standard solution of omeprazole (100 µg/ml) was prepared by dissolving 10 mg of the pure drug in 100 ml methanol in a volumetric flask.

General procedures for the determination of omeprazole through nitrosation reaction

Accurately measured aliquots of standard solution containing (0.2-1.1 mg/ml) of omeprazole was transferred into a series of 10 ml volumetric flasks. To each flask, 0.5 ml of 10% *w/v* sodium nitrite and 1 ml of 0.1% *v/v* HNO_3 were added. The contents of the flasks were mixed well.

The solution was cooled in ice bath (0-5°C) for 10 minutes then 2 ml of 0.1 M NaOH was added. The mixture was diluted to 10 ml with distilled water. The absorbance of the yellow colored solution was measured at λ_{max} 390 nm against the blank. Fig. 2

Pharmaceutical preparation (capsules)

The contents of twenty capsules of Pepzol® were emptied and pulverized and accurately weighed amount equivalent to concentration of omeprazole taken in the method were extracted by shaking with 20 ml methanol three times, the filtrates were collected and transferred to 100 ml volumetric flask, completed to the mark with distilled water. Aliquots from this solution equivalent to that in authentic sample were used for the application of the proposed method applying standard addition technique.

RESULTS AND DISCUSSION

Omeprazole molecule undergoes aromatic C-nitrosation by nitrite. The dominant reaction was C-4, C-6 nitrosation through a mechanism that appears to consist of an electrophilic attack on the nitrosable substrate by $H_2NO_2^+$\NO^+, followed by slow proton transfer [15].

Yellow colored solution is produced measured at λ_{max} 390 nm. Methoxy group at position 5 activates electrophilic substitution in C-4, C-6 positions through its mesomeric effect. The suggested mechanism is explained below. Scheme No.1

Study of the experimental parameters

i-Effect of reagent concentration

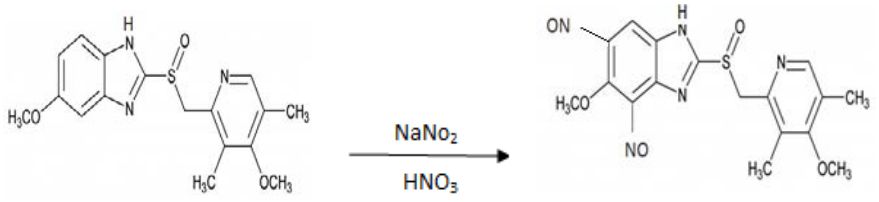
Addition of 0.5 ml of 10% *w/v* $NaNO_2$ and 1 ml of 0.1% *v/v* HNO_3 were sufficient to obtain the maximum and reproducible absorbance values for the various concentration ranges of omeprazole. Fig. 3- 6

ii-Effects of temperature and reaction time

The effects of temperature and reaction time on the formation of the colored complex were also optimized. The color intensity increased upon leaving the flask content in ice bath for10 minutes. Fig. 7

iii-Effect of NaOH concentration

The amount of NaOH solution for maximum color intensity was examined. The maximum constant color intensity was reached on using 2 ml of 0.1M NaOH solution. Fig. 8



Scheme 1: Proposed reaction scheme between omeprazole and nitrous acid

Table 1: Statistical data for the determination of omeprazole through nitrosation reaction

Parameters	Conc. taken µg/ml	Conc. found µg/ml	Recovery* %
	20	19.92	99.63
	40	40.07	100.18
	60	60.50	100.84
	80	80.65	100.81
	100	99.49	99.49
	110	110.36	100.32
Mean recovery*			100.22
N			6
Beer's law			20-110 µg/ml
SD			0.570
RSD			0.569
SE			0.233
Variance			0.325
LOD, µg ml^{-1}			5.21
LOQ, µg ml^{-1}			15.79
Sandell's sensitivity (µg cm^{-2}**)**			1.89 x 10^{-2}
Apparent Molar absorbitivity** L Mol^{-1} cm^{-1}			2.477 x10^{3}

*Mean of three different experiments. **Calculated in the basis of molecular weight of the drug.

Table 2: Statistical data for determination of pharmaceutical capsules of omeprazole by standard addition technique and comparing the results with the reference method [17]

Items	Conc. added form pure drug (µg/ml)	Conc. taken from Pepzol® (µg/ml)	Conc. found (µg/ml)	Recovery*%
	20	0	20.14	100.71
	20	30	49.71	99.42
	20	40	60.43	100.71
	20	50	69.86	99.79
	20	60	80.29	100.35
Mean recovery*				100.16
N				5
S.D.				0.653
R.S.D.				0.652
V				0.427
S.E.				0.29
t-test**				0.374
F-test**				1.149

*Mean of three different experiments. **Theoretical t and F values are 20306 and 5.05, respectively at p=0.05.

Validation of the proposed method

The validity of the proposed methods was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendations [16].

Linearity and range

The calibration graph obtained by plotting the values of the absorbance versus the final concentrations (µg/ml) was found to be rectilinear over the concentration ranges cited in the table 1.

Correlation coefficient, intercept and slope for the calibration data are summarized in table 1.

Limits of detection and limits of quantification

Limits of detection (LOD) were determined by evaluating the lowest concentrations of the analyte that can be detected according to the following equation: LOD = 3.3S/K

Limits of quantification (LOQ) were determined also by establishing the lowest concentrations that can be detected according to the following equation: 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. The results are summarized in table 1.

Accuracy and interference liabilities

The accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique. The results are shown in table 2. Before proceeding with the analysis of omeprazole in capsules, interference liabilities were carried out to explore the effect of common excipients that might be added during the capsule formulation. Samples were prepared by mixing known amount of omeprazole with 50 mg talc, 50 mg starch and 50 mg mannitol. These laboratory prepared samples were analyzed by the proposed methods. 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. The intraday SD values revealed the precision of the methods (values vary from 0.158 to 0.893). **For inter - day reproducibility**, a series was run, in which the standard drug solutions were analyzed each for five days. The inter-day SD values were in the range of 0.495 – 0.938. The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable.

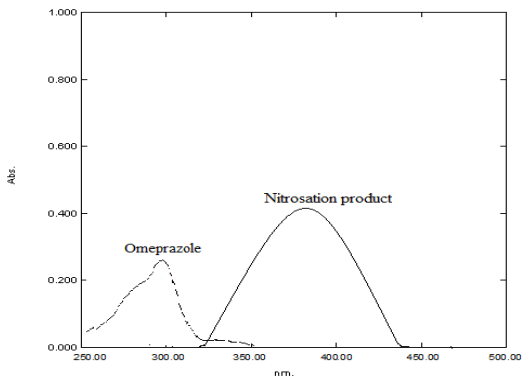


Fig. 2: Absorption spectrum for the nitrosation reaction of omeprazole (60 µg ml^{-1}) at λ_{max} 390 nm.

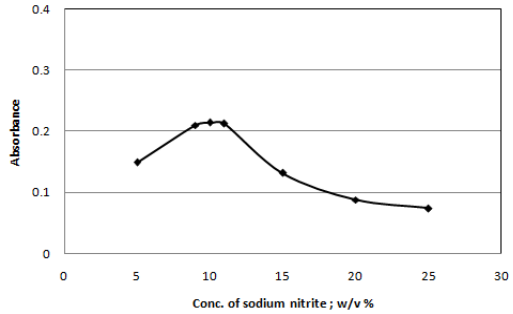


Fig. 3: Effect of concentration of sodium nitrite on the nitrosation reaction with omeprazole (30 µg ml^{-1})

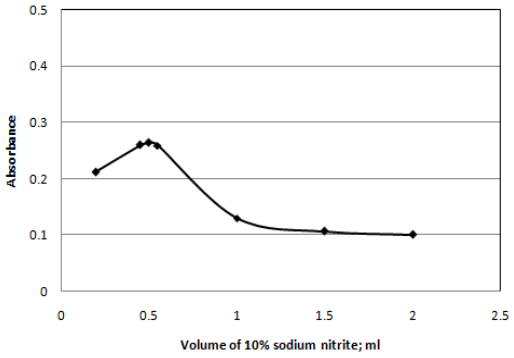


Fig. 4: Effect of volume of sodium nitrite on the nitrosation reaction with omeprazole (30 µg ml^{-1}).

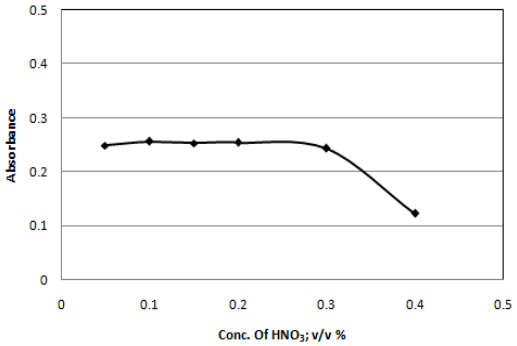


Fig. 5: Effect of concentration of HNO_3 on the reaction with omeprazole (30 µg ml^{-1}).

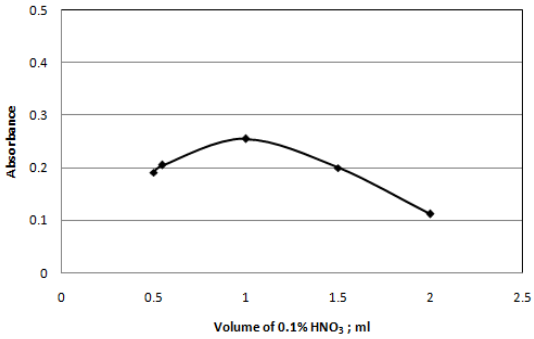


Fig. 6: Effect of volume of HNO_3 on the reaction with omeprazole (30 µg ml^{-1}).

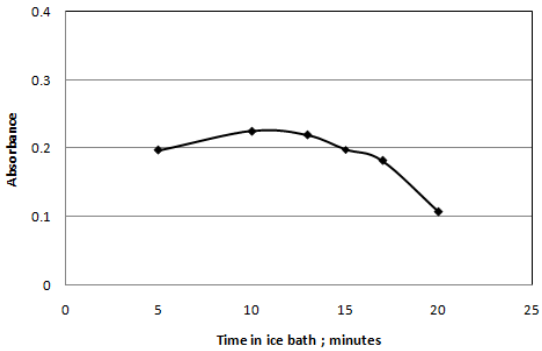


Fig. 7: Effect of cooling time at (0-5°C) on the nitrosation reaction with omeprazole (30 µg ml^{-1}).

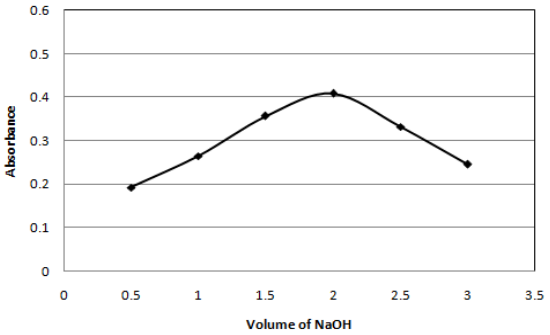


Fig. 8: Effect of volume of 0.1M NaOH on increasing color intensity after nitrosation reaction

Robustness

Robustness of the method was examined by small changes in the method variables such as change reagent concentration (±0.2 ml), pH of buffer solution (±0.2) and reaction time (±5 minutes). The minor changes that may take place during the experiment didn't affect the absorbance of the reaction products.

Analysis of capsules

The proposed methods were applied to the analysis of the drug in capsules and the results were statistically compared with reference method [17] by calculating Student's *t*- and F-values. The evaluated *t*- and F-values were less than the tabulated values at the 95% confidence level. The results are listed in table 2.

CONCLUSION

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

ACKNOWLEDGEMENT

All authors are thankful to Faculty of Pharmacy, Zagazig University, P.C. 44519, Zagazig, Egypt for providing facility to carry out the research work.

REFERENCES

1. M. H, United F. The British Pharmacopoeia", Office, London2011.
2. "The United States Pharmacopoeia", U.S.P. The National Pharmacopeial Convention, Inc. Chem Pharm Bull (Tokyo) 2010;32.
3. Sweetman SC. "Martindale-The Complete Drug Reference", 37th edition, The Pharmaceutical Press, London 2011.
4. Syed AA, Syeda A. Spectrophotometric determination of certain benzimidazole proton pump inhibitors. Indian J Pharm Sci 2008;70(4):507-10.
5. Ashraf M. Mahmoud, New sensitive kinetic spectrophotometric methods for determination of omeprazole in dosage forms Int J Anal Chem, 2009, 2009, 1-11.
6. Lotfy HM, Abdel-Monem Hagazy M. Comparative study of novel spectrophotometric methods manipulating ratio spectra:an application on pharmaceutical ternary mixture of omeprazole, tinidazole and clarithromycin. Spectrochimica acta Part A, Molecular and biomolecular spectroscopy 2012;96:259-70.
7. Shaghaghi 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(4):256-62.
8. El-Sherif ZA, Mohamed AO, El-Bardicy MG, El-Tarras MF. Reversed-phase high performance liquid chromatographic method for the determination of lansoprazole, omeprazole and pantoprazole sodium sesquihydrate in presence of their Acid-induced degradation products. J Chem Pharm Bull (Tokyo) 2006;54(6):814-8.
9. Ariane P, Rafael N, Bruno R, Marcos AS. Fábio S. Murakami Cruz Pereira ValenteSilva Development and validation of a RPHPLC method to quantify omeprazole in delayed release tablets. J Liquid Chromatography and Related Tecnologies 2007;30(1):113-21.
10. Darwish KM, Salama I, Mostafa S, El-Sadek M. RP-HPLC/pre-column derivatization for analysis of omeprazole, tinidazole, doxycycline and clarithromycin. J Chromatogr Sci 2013;51(6):566-76.
11. Raval PB, Puranik M, Wadher SJ, Yeole PG. A Validated HPTLC Method for Determination of Ondansetron in Combination with Omeprazole or Rabeprazole in Solid Dosage Form. Indian J Pharm Sci 2008;70(3):386-90.
12. Berzas NJJ. Castañeda P. G Rodrguez D RM Method development and validation for the separation and determination of omeprazole enantiomers in pharmaceutical preparations by capillary electrophoresis. J Anal Chim Acta 533;2005:127-33.
13. J. Jin-long Yan, Electrochemical behavior and the determination of omeprazole using glassy carbon electrode. J Sci Chem Pharm Bull (Tokyo) 2006;6(7):1625-7.
14. Qaisi AM, Tutunji MF, Tutunji LF. Acid decomposition of omeprazole in the absence of thiol:a differential pulse polarographic study at the static mercury drop electrode (SMDE). J Pharm Sci 2006;95(2):384-91.
15. Mario G, Jorge A, Emilio C, Julio C, Aromatic C. nitrosation of a bioactive molecule. Nitrosation of minoxidil Organic and Biomolecular Chemistry 9;2011:7680-4.
16. Tripartite U, H. IC. International Conference On Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Validation of Analytical Procedures:Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November incorporated in November , London. J Chem Pharm Bull (Tokyo) 2005;1996.
17. Bhandage A, Bhosale A, Kasture A, Godse VP, Trop P, J. Extractive Spectrophotometric Determination of Omeprazole in Pharmaceutical. J Chem Pharm Bull (Tokyo) 2009;8(5):449-54.