

## Indirect spectrofluorimetric determination of omeprazole by its quenching effect on the fluorescence of Tb<sup>3+</sup>-1,10-phenanthroline complex in presence of bis (2-ethylhexyl) sulfosuccinate sodium in capsule formulations

<sup>1</sup>Shaghghi M., <sup>\*1</sup>Manzoori J.L., <sup>2</sup>Jouyban A.

<sup>1</sup>Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz, <sup>2</sup>Faculty of Pharmacy and Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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### ABSTRACT

**Purpose:** Omeprazole (OMZ) is a substituted benzimidazole, which is used in the treatment of gastric acid related disorders. The aim of this study was the development and validation of a rapid, simple and reliable fluorimetric method for determination of OMZ in pharmaceutical formulations based on fluorescence quenching of Tb<sup>3+</sup>-1, 10-phenanthroline complex.

**Method:** For the determination of OMZ, aliquots of Tb<sup>3+</sup>, bis (2-ethylhexyl) sulfosuccinate sodium (AOT), 1,10-phenanthroline (phen) solutions (in optimal concentrations), aliquots of working OMZ solution and Tris-HCl buffer (pH 7.0) solution were added to 5 mL volumetric flasks. The mixture was then diluted with distilled water and allowed to stand for 30 min and the fluorescence intensity was then measured at 545 nm using an excitation wavelength of 300 nm. Matrix systems of OMZ (OMZ capsules with a nominal of 20 mg) were prepared by powdering and mixing the contents of ten capsules of OMZ. A portion of 10.0 mg of this powder was then accurately weighed and dissolved in about 10 mL of 0.1 M NaOH solution and filtered into a 100 mL volumetric flask. The residue was washed several times with water and solution was diluted to the mark. A suitable aliquot of this solution was applied for fluorimetric determination of OMZ. The recovery assay was carried out using the same procedure by addition of known amounts of OMZ.

**Results:** It was found that the fluorescence intensity of Tb<sup>3+</sup>-1, 10-phenanthroline complex can be greatly quenched by omeprazole in the presence of AOT. Under optimal conditions, the quenched fluorescence intensity was found to be proportional to the concentration of omeprazole in the range of 0.05-10 µg/mL. The detection limit was 0.016 µg/mL. The relative standard deviation values for 6 replicated determinations of 0.3 and 1.5 µg/mL of OMZ were 3.5 and 1.5 %, respectively, The RSD of intraday was 2.6 and that of interday was 3.4 % for 4 and 2 µg/mL of OMZ, respectively.

**Conclusion:** Based on the obtained results, a simple, rapid and selective spectrofluorimetric method was developed for determination of omeprazole in its capsule formulations with excellent reproducibility and allowed the interference-free determination of OMZ in real samples.

**Keywords:** Sensitized fluorescence, Omeprazole, Terbium, Fluorescence quenching

### INTRODUCTION

Omeprazole (OMZ) (figure 1) (5-methoxy-2-[[[4-methoxy-3, 5-dimethyl-2-pyridinyl] methyl] sulfinyl]-1H-benzimidazole), which is used in the treatment of gastric acid related disorders (1). It is decomposed in acid media to yield two main products, i.e. sulphonamide and sulphenic acid. OMZ has also been found to be unstable in neutral and weak alkaline media but is stable in strong

alkaline solutions where the maximum stability is at pH 11.

Several high-performance liquid chromatography (HPLC) methods with ultraviolet detection and electrochemical detection (2-5), liquid chromatography coupled with tandem mass spectrometry (6), spectrophotometry (7-10), polarography (11-13), voltammetry (14), capillary electrophoresis (15) and thin-layer chromatography (16) methods

have been developed for determination of omeprazole in different samples. However these methods suffer from a number of limitations such as complex automated sample preparation system (4), liquid-liquid or uneconomical solid-phase extraction (SPE) (2-6), inconvenient chromatography (2), long analysis times (3), or the use of toxic halogenated solvents during sample preparation (3).

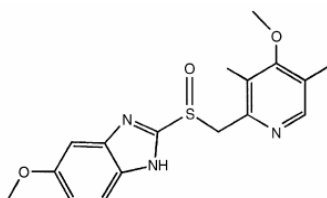


Figure 1. Structure of omeprazole.

Because of the high sensitivity and selectivity, and being rapid and simple, spectrofluorimetric methods have been widely used for determination of a wide range of pharmaceuticals with the advantages that in most instances, derivatization is not required, and that these methods are less sensitive to matrix effects than other analytical techniques (17, 18).

Rare-earth ions have luminescence characteristics such as narrow spectral width, long luminescence lifetime and large Stokes shift. Therefore, the rare earth  $Tb^{3+}$  is often used as a fluorescence probe for determination of some substances because of the high fluorescence quantum efficiency of the terbium (III) chelates (18, 19).

A previous study (20) demonstrated that  $Tb^{3+}$ -1,10-phenanthroline (phen) complex had a strong intrinsic fluorescence of  $Tb^{3+}$  and since it was found that its fluorescence could be quenched by OMZ, a novel method for determination of OMZ was developed. To the best of our knowledge, this is the first attempt to determine OMZ in capsules by spectrofluorimetry.

## MATERIALS AND METHODS

### Reagents

All reagents and solvents were analytical grades and used without further purification. Doubly distilled water was used throughout this work. Two commercial OMZ products were purchased from a local pharmacy.

A  $10^{-2}$  M terbium (III) solution was prepared by dissolving the appropriate amount of terbium (III) chloride hexahydrate ( $TbCl_3 \cdot 6 H_2O$ ) (Acros Organics, USA) in doubly distilled water and stored in a polyethylene containers to avoid memory effects of terbium adsorbed on glass vessels.

OMZ powder was provided by Exir pharmaceutical company (Lorestan, Iran). A stock standard solution of 100  $\mu g/mL$  was prepared by dissolving 10.0 mg of OMZ in approximately 10 mL of 0.1 M sodium hydroxide and was diluted to the mark in 100 mL volumetric flask with water.

A  $10^{-2}$  M stock solution of 1, 10-phenanthroline (Fluka, Switzerland) was prepared in 10 mL ethanol and was diluted to the mark in 100 mL volumetric flask with doubly distilled water. A  $10^{-2}$  M solution of bis (2-ethylhexyl) sulfosuccinate sodium (AOT) (Fluka, Switzerland) solution was prepared by dissolving the appropriate amount of AOT in doubly distilled water. Also aqueous solutions of N-cetyl-N,N,N-trimethylammonium bromide (CTAB), polyoxyethylene lauryl ether (Brij-35), sodium dodecyl sulfate (SDS), Triton X-100, hydrochloric acid, sodium hydroxide,  $\alpha$  and  $\beta$ -cyclodextrin (all were obtained from Merck) were prepared.

A 0.05 M Tris-(hydroxymethyl) aminomethan-hydrochloric acid (Tris- HCl) buffer solution was prepared by dissolving a desired amount of Tris-base (Merck) in 90 mL of water, adjusting the pH to 7.0 with HCl and making up the volume to 100 mL with water. Working standard solutions were prepared daily by successive dilution of the stock standard with water.

### Apparatus

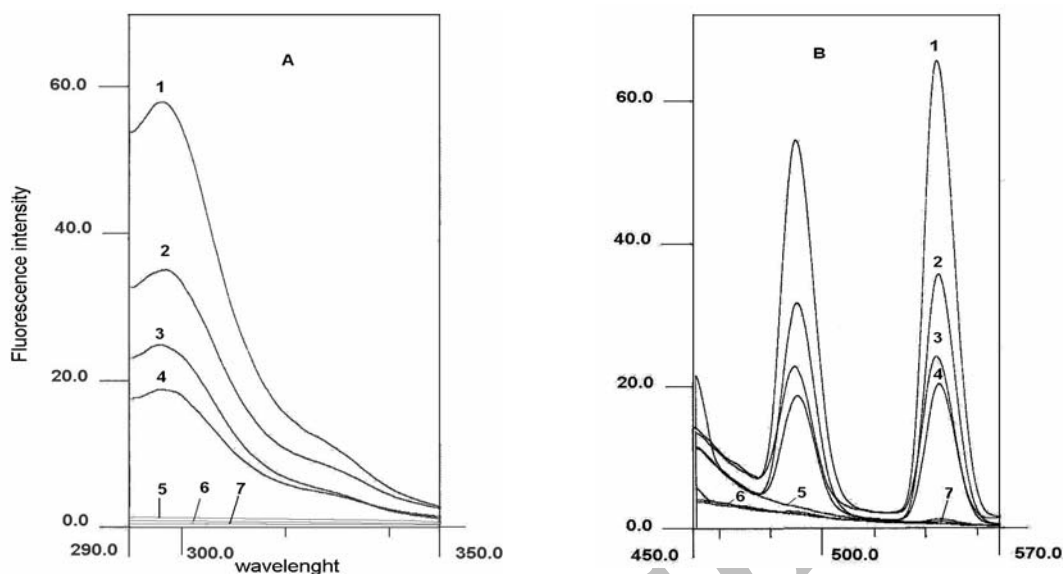
Fluorescence spectra and intensity measurements were performed using a Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan) equipped with a 150 W xenon lamp, using 1.0 cm quartz cell. The excitation and emission monochromator bandwidths were 10 nm. The excitation wavelength was set at 300 nm and the fluorescence was measured using the peak height at 545 nm. All measurements were performed at  $25 \pm 0.1$  °C using a thermostated cell holder and a thermostatically controlled water bath (Rikakika, Japan). The pH of solutions was measured using Metrohm model 654 pH meter (Herisau, Switzerland).

### Methods

All measurements were corrected for the background fluorescence of blank which was taken as the solution containing all reagents except OMZ. Optimization of terbium sensitized fluorescence was investigated by variation of the pH or the concentration of one of the components while the remaining factors were kept constant.

### Experimental procedure

For determination of OMZ, the analytical procedure which was used to construct the calibration graph was as follow: to 5 mL



**Figure 2.** Terbium-sensitized fluorescence excitation ( $\lambda_{em} = 545$  nm) (A) and emission ( $\lambda_{ex} = 300$  nm) (B) spectra: (1) Tb-phen-AOT; (2) Tb-phen; (3) Tb-phen-OMZ; (4) Tb-phen-AOT-OMZ; (5)  $Tb^{3+}$ ; (6) OMZ; (7) AOT. Conditions:  $[Tb^{3+}] = 2 \times 10^{-4}$  M,  $[OMZ] = 2$   $\mu$ g/mL,  $[AOT] = 10^{-4}$  M, (pH = 7.0).

volumetric flasks, 1 mL of  $1 \times 10^{-3}$  M  $Tb^{3+}$  solution, 50  $\mu$ L of  $1 \times 10^{-2}$  M AOT solution, 0.5 mL of  $1 \times 10^{-3}$  M phen solution, aliquots of working OMZ solution and 2 mL of 0.05 M Tris-HCl buffer (pH 7.0) solution were added. The mixture was then diluted to the mark with distilled water and allowed to stand for 30 min. The final OMZ concentration was in the range of 0.05-10  $\mu$ g/mL. The solutions were thermostated at  $25 \pm 0.1$  °C and the fluorescence intensity was measured at 545 nm using an excitation wavelength of 300 nm against a blank solution. Both emission and excitation slits were set at 10 nm. The quenched fluorescence intensity of  $Tb^{3+}$ -phen-AOT by OMZ was represented as  $\Delta I_f$  (%) =  $(I_0 - I_f)/I_0 \times 100$  in which  $I_f$  and  $I_0$  were the intensities of the systems with and without OMZ, respectively.

#### Pharmaceutical preparation

Ten capsules of OMZ were weighed in order to find the average mass of each capsule. Then the contents were powdered and mixed. A portion of 10.0 mg of this powder was accurately weighed and dissolved in about 10 mL of 0.1 M NaOH solution and filtered into a 100 mL volumetric flask. The residue was washed several times with water and solution was diluted to the mark. A suitable aliquot of this solution was taken for fluorimetric determination of OMZ. The recovery assay was carried out using the same procedure by addition of known amounts of OMZ.

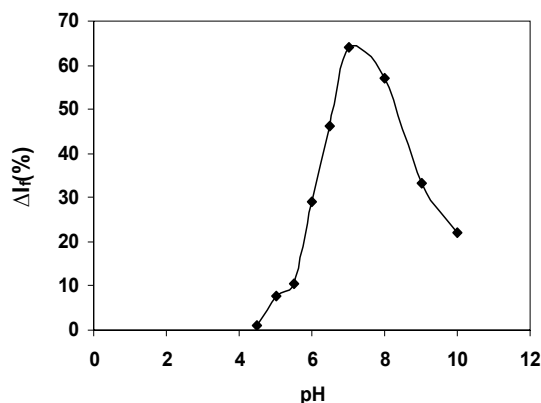
## RESULTS AND DISCUSSION

### Fluorescence spectra

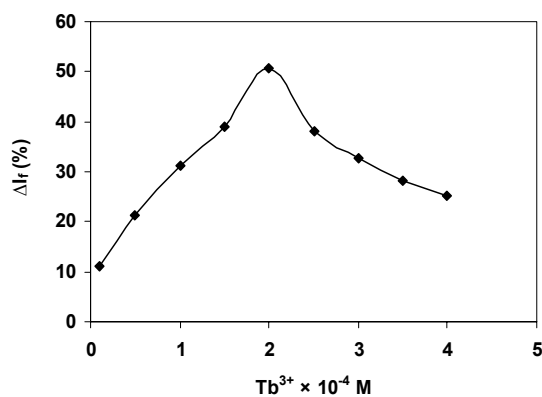
Fluorescence excitation and emission spectra of (1) Tb-phen-AOT, (2) Tb-phen, (3) Tb-phen-OMZ, (4) Tb-phen-AOT-OMZ, (5)  $Tb^{3+}$ , (6) OMZ and (7) AOT systems at pH 7.0 are shown in figure 2. From spectra 5 (B) in figure 2, it may be found that almost no characteristic fluorescence of  $Tb^{3+}$  was observed in the  $Tb^{3+}$  system. It was found that after the excitation of 300 nm, the  $Tb^{3+}$ -phen system (3) emitted the characteristic fluorescence of  $Tb^{3+}$  ion with the emission peaks of 490 and 545 nm, which correspond to  $^5D_4 \rightarrow ^7F_6$  and  $^5D_4 \rightarrow ^7F_5$  transitions of  $Tb^{3+}$ , respectively. The addition of OMZ could quench fluorescence intensity of  $Tb^{3+}$ -phen system. However, in the presence of AOT, the fluorescence of this system was greatly quenched by OMZ, which indicated that there was interaction between OMZ, AOT and  $Tb^{3+}$ -phen. Therefore the  $Tb^{3+}$ -phen-AOT system was utilized in the assays of OMZ. Since the quenched fluorescence intensity at 545 nm was higher, this wavelength was chosen to detect the fluorescence intensities throughout all the experiments.

### Factors affecting the fluorescence intensity of the system

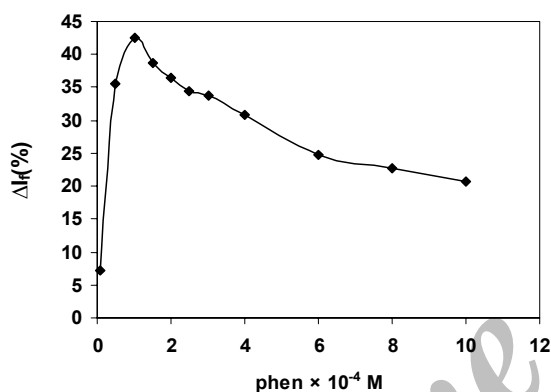
The effect pH on the quenched fluorescence intensity of the  $Tb^{3+}$ -phen-OMZ-AOT system was investigated in the pH range of 4.5-10.0 (figure 3). The results indicated that when the  $Tb^{3+}$ -phen complex was more stable, OMZ had little effect



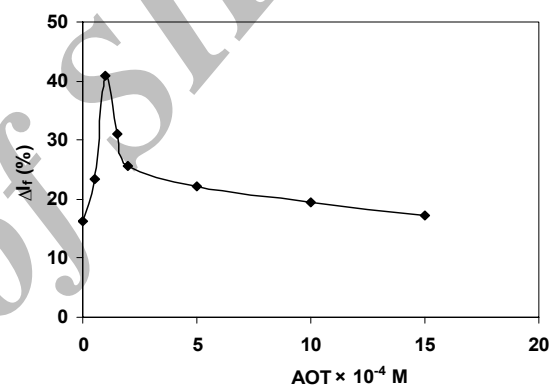
**Figure 3.** Effect of pH. Conditions: phen,  $2 \times 10^{-4}$  M;  $Tb^{3+}$ ,  $10^{-4}$  M; AOT,  $10^{-4}$  M; OMP, 2  $\mu$ g/mL.



**Figure 4.** Effect of  $Tb^{3+}$  concentration. Conditions: phen,  $2 \times 10^{-4}$  M; AOT,  $10^{-4}$  M; OMP, 2  $\mu$ g/mL, Tris-HCl, 0.02 M (pH = 7.0).



**Figure 5.** Effect of phen concentration. Conditions:  $Tb^{3+}$ ,  $2 \times 10^{-4}$  M; AOT,  $10^{-4}$  M; OMP, 2  $\mu$ g/mL; Tris-HCl, 0.02 M (pH = 7.0).



**Figure 6.** Effect of AOT concentration. Conditions:  $Tb^{3+}$ ,  $2 \times 10^{-4}$  M; phen,  $10^{-4}$  M; OMP, 2  $\mu$ g/mL; Tris-HCl, 0.02 M (pH = 7.0).

on the complex (in the pH range of 2.0-5.5), and the quenched fluorescence intensity of the system reached a maximum when pH was 7.0. At higher pH (pH >7.0) the intensity decreased due to the precipitation of terbium hydroxide.

The results of the effect of the concentration of  $Tb^{3+}$  are shown in figure 4. The quenched fluorescence intensity of the system reached a maximum when  $Tb^{3+}$  concentration was  $2 \times 10^{-4}$  M. Therefore,  $2 \times 10^{-4}$  M of  $Tb^{3+}$  was chosen for further investigation.

The effect of phen concentration on the fluorescence intensity of  $Tb^{3+}$ -phen-AOT-OMZ system was studied (see figure 5), and was found that the quenched fluorescence intensity of  $Tb^{3+}$ -phen-AOT-OMZ system reached a maximum when the concentration of phen was  $1 \times 10^{-4}$  M.

The effects of the following surfactants and cyclodextrins (CDs) on the fluorescence intensities were tested. The  $\Delta I_f$  (%) for AOT, sodium dodecyl sulphonate (SDS), cetyltri-

methylammonium bromide (CTAB), Triton X-100,  $\alpha$ -CD,  $\beta$ -CD and Brij 35 were 51.1, 43.7, 27.8, 18.6, 37.5, 25 and 18.4, respectively. The  $\Delta I_f$  (%) of the system without surfactants was 16.2, which was the lowest in above systems. This indicated that addition of surfactants and/or CDs increase the quenching effect of OMZ on the fluorescence of  $Tb^{3+}$ -phen complex. The results showed that AOT had the best quenching effect on the fluorescence intensity of the system. The main reasons seem to be its negative charges and little steric hindrance. The effect of the AOT concentration was studied and is shown in figure 6. It was found that the  $\Delta I_f$  (%) of this system reached a maximum when the concentration of AOT was  $1 \times 10^{-4}$  M.

The influence of the order of addition on the fluorescence intensity of this system was investigated and it was found that the best order of addition was  $Tb^{3+}$ -AOT-phen-OMZ-Tris-HCl and this order was selected for further investigations. Under optimal condition, the effect of time on the

**Table 1.** Tolerance limits of various interferents in the determination of 2 µg/mL of OMZ

Substances	Interferent-to-analyte ratio <sup>(a)</sup>	Change of $\Delta I_f$ (%)
Mannitol	60:1	-4.9
Lactose anhydrous	13:1	-3.8
Maize starch	10:1	3.3
Macrogol (PEG 6000)	14:1	1.7
Sodium carboxymethyl cellulose (NaCMC)	7:1	-3.7
Gelatin	56:1	-1.4
Povidone (PVP 25000)	7:1	2.5
Sodium laurylsulfate (SLS)	7:1	-6.3
Hypromellose (HPC)	4:1	2.6
Hypromellose phthalate (HPMC phthalate)	0.4:1	-7.0
Cellulose microcrystalline	0.7:1	-2.5
Magnesium stearate	0.4:1	-3.1
Disodium hydrogen phosphate dihydrate	0.07:1	5.1

<sup>(a)</sup> Interferent-to-analyte ratio is in final solutions.

**Table 2.** Determination of OMZ in pharmaceutical preparations (20 mg capsules)

	EXi PRAZOLE <sup>®</sup> 20	OMEPRAZOLE 20
Found $\pm$ s (mg per capsule) <sup>a</sup>	19.71 $\pm$ 0.17 t = 3 (4.3) <sup>c</sup> (n = 3)	21.3 $\pm$ 1.1 t = 2 (4.3) (n = 3)
Recovery $\pm$ s (%) <sup>b</sup>	98.5 $\pm$ 0.84	106.3 $\pm$ 5.6

<sup>a</sup> Standard deviation (average of three determination).

<sup>b</sup> Recovery is calculated from the content reported by laboratory.

<sup>c</sup> The figure in parentheses is the tabulated t value at 95% confidence level.

**Table 3.** Recoveries of OMZ added to the pharmaceutical preparations

Amount added (µg/mL) <sup>a</sup>	Amount found (µg/mL) <sup>b</sup>	
	EXi PRAZOL <sup>®</sup> 20	OMEPRAZOLE 20
4.0	4.1 $\pm$ 0.12, (102.5 $\pm$ 3.1) <sup>c</sup>	-
6.0	6.1 $\pm$ 0.21, (101.7 $\pm$ 3.3)	-
8.0	8.2 $\pm$ 0.34, (102.5 $\pm$ 4.3)	-
2.0	-	1.9 $\pm$ 0.058, (95.0 $\pm$ 2.9)
4.0	-	4.3 $\pm$ 0.098, (107.5 $\pm$ 2.5)
6.0	-	6.4 $\pm$ 0.18, (106.7 $\pm$ 3.0)

<sup>a</sup> The given values are concentration in prepared samples.

<sup>b</sup> Average of three determinations  $\pm$  S.D.

<sup>c</sup> Recovery (%).

fluorescence intensity was studied. After addition of all reagents, oscillated for 5 min and allowed to stand for 25 min, the  $\Delta I_f$  (%) reached a maximum and remained basically stable for over 1 h without having any significant effects on the results.

#### Analytical figures of merit

By using the optimized conditions described above, a spectrofluorimetric method was developed for determination of OMZ in the capsules. The calibration graph (n = 12) was found to be linear in the range of 0.05 to 10 µg/mL for OMZ and its equation was  $\Delta I_f$  (%) = 6.57 C + 15.27, where  $\Delta I_f$  (%) is the quenched

fluorescence intensity of Tb<sup>3+</sup>-phen-AOT by OMZ and C is the concentration of OMZ expressed in µg/mL.

The limit of detection was calculated as  $3S_b/m$  (where  $S_b$  is standard deviation of the blank and m is slope of the calibration graph) and it was found to be 0.016 µg/mL.

In order to study the precision of the proposed method (repeatability), series of six solutions of 0.3 and 1.5 µg/mL of OMZ were measured on the same day. By applying the ICH definition, the relative standard deviation (RSD) for six analyses were 3.5 and 1.5 %, respectively. To assess the day-to-day precision (intermediate precision),

repeated analyses of 4 and 2  $\mu\text{g/mL}$  of OMZ (six analyses) were performed over two weeks and interday RSD were 2.6 and 3.4 %, respectively. The selectivity of the method was investigated and no interferences were observed between OMZ and common excipients for capsules formulations. The interference of typical excipients (21) was studied by addition of increasing concentration of these compounds to solution of 2  $\mu\text{g/mL}$  of OMZ until variation greater than 5 % in analytical intensity was achieved. As shown in the Table 1, most excipients except disodium hydrogen phosphate dehydrate and hypromellose phthalate (due to interaction of phosphate and phthalate by terbium, [22, 23]) either had no effect or had little effect on the determination of OMZ under the permission of  $\pm 5$  % relative error. Hence selectivity achieved by the proposed method is good and it is possible to determine OMZ in the presence of the excipients.

#### Application

OMZ was satisfactorily analyzed in two commercial products of Iranian pharmaceutical market (OMZ capsules with a nominal of 20 mg) by terbium sensitized fluorescence method. According to the characteristics of the fluorescence spectra of these two preparations, there was no interference from the excipients. Table 2 shows the results for triplicate analyses. Statistical analysis of the assay results showed satisfactory accuracy and precision of the proposed method with no significant differences between certified and experimental results ( $P < 0.05$ , Table 2).

Recovery of OMZ from pharmaceutical preparations spiked with different amounts of this drug was between 95 % and 107.5 %. (Table 3). The RSDs of the method for triplicate analyses of capsule samples containing different brands of OMZ were 2.3 - 4.1%.

#### Mechanism of Interaction of the system

As it can be found from figure 1, in the presence of AOT, OMZ quenches the fluorescence of  $\text{Tb}^{3+}$ -

phen system, and there is an interaction among OMZ, AOT and  $\text{Tb}^{3+}$ -phen which may be a large complex of  $\text{Tb}^{3+}$ -phen-AOT-OMZ in this system. A previous investigation (20) has shown that  $\text{Tb}^{3+}$  can combine with phen to form 1:3 complexes with positive charge, which emits strong characteristic fluorescence of  $\text{Tb}^{3+}$ . This is attributed to intramolecular energy transfer between phen and  $\text{Tb}^{3+}$ . On the other hand, OMZ has coordination ability towards some cations (10). Structural formulae of OMZ, showed that metal chelates can be formed between the imidazole-NH group, the oxygen of the sulfur dioxide side chain, and the N of the pyridine to yield a six-membered ring. Therefore, OMZ may coordinates with  $\text{Tb}^{3+}$  in Tb-phen system. On the other hand, AOT with negative charges can react with  $\text{Tb}^{3+}$ -phen complex through electrostatic forces. Therefore, both AOT and OMZ can react with  $\text{Tb}^{3+}$ -phen complex, which weaken combination between  $\text{Tb}^{3+}$  and phen resulting in the fluorescence quench of  $\text{Tb}^{3+}$ -phen system.

#### CONCLUSIONS

The official analytical method of OMZ in BP is the non-selective titrimetric method with standard NaOH (24). The conventional UV method (9) suffer from interference due to UV absorbing compounds in the determination of OMZ.

On this basis, of results of this study a new spectrofluorimetric method was developed for determination of OMZ in real samples based on terbium sensitized fluorescence. The proposed method is very simple, precise, and rapid was easily applied to determination of OMZ in capsules with excellent reproducibility and allowed the interference-free determination of OMZ in real samples.

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