

Original Article

A VALIDATED SPECTROFLUORIMETRIC METHOD FOR THE ASSAY OF SOME PROTON PUMP INHIBITORS USING SODIUM 1, 2-NAPHTHOQUINONE-4-SULPHONATE

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Received: 07 Mar 2014 Revised and Accepted: 27 Mar 2014

ABSTRACT

A simple, rapid and sensitive spectrofluorimetric method was developed for the determination of certain proton pump inhibitors (PPIs) belonging to benzimidazole drugs omeprazole (OMZ), rabeprazole (RAB), pantoprazole (PAN) and lansoprazole (LAN). The method depends on coupling with sodium 1, 2-naphthoquinone-4-sulphonate (NQS) in presence of methanolic solution of iodine and alkaline medium to yield an intensely fluorescent derivative measured at $\lambda_{exc.} = 340$ nm and $\lambda_{em.} = 480$ nm. A linear relationship was achieved between measured relative fluorescence intensity and concentration of each of the studied drug in the ranges 5-60, 10-80, 10-65 and 10-85 ng ml⁻¹ for OMZ, LAN, PAN and RAB, respectively with good correlation coefficients. The limits of detection and quantification, intra-day, inter-day precision, and accuracy of the method have been evaluated. The proposed method was successfully applied to the assay of the studied PPIs in dosage forms and the results were statistically agree well with those of reported methods.

Keywords: Proton-pump inhibitors, Spectrofluorometry, 1,2-Naphthoquinone-4-Sulphonate, Iodine, Sodium hydroxide, Pharmaceutical analysis.

INTRODUCTION

Omeprazole, rabeprazole, pantoprazole and lansoprazole are benzimidazole derivatives which are used in the treatment of gastric and duodenal ulcers, and reflux oesophagitis [1], Fig.1. Their efficacy as antiulcer and anti-secretory agents have been well established.

A literature survey reveals that reported methods used for determination of the cited drugs in pharmaceutical dosage forms and biological fluids include titrimetry [2, 3], UV- VIS spectrophotometry [4-17], high performance thin-layer chromatography [18-24], capillary electrophoresis [7] and electrochemical methods [36,37]. Spectrofluorometric methods are widely used due to their sensitivity and simplicity.

Few Spectrofluorimetric methods were reported for analysis of some PPIs in pure forms, in pharmaceutical dosage forms and in presence of impurities which mainly depends on quenching effect or direct measurement [5, 38, 39]. Coupling of NQS with compounds containing active methylene group, followed by reduction with potassium borohydride to give fluorescent derivative was previously reported [40]. In the present investigation, a novel modification of this reacting was introduced by allowing the ppls to interact via their active methylene groups with NQS in the presence of iodine. Iodine aids the process of desulphonation of NQS in the course of reaction and to facilitate the coupling process; and an intensely fluorescent.

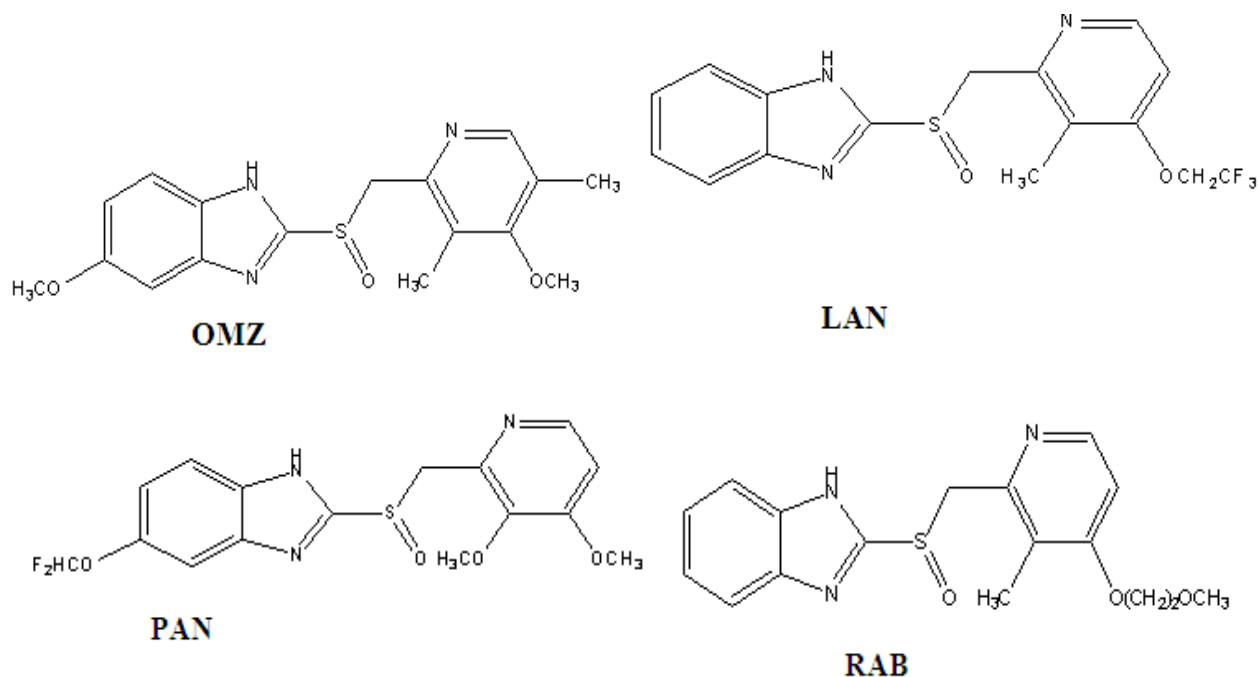


Fig. 1: Chemical structures of the studied proton pump inhibitors.

derivative was obtained without need for reduction by potassium borohydride. The aim of the present work is to develop a new and sensitive spectrofluorimetric method for the assay of the four studied PPIs based on their coupling with sodium 1,2-naphthoquinone-4-sulphonate (NQS) in presence of methanolic solution of iodine and alkaline medium to yield an intensely fluorescent derivative at $\lambda_{exc.} = 340$ nm and $\lambda_{em.} = 480$ nm.

MATERIALS AND METHODS

Apparatus

A Shimadzu RF-5301 PC spectrofluorophotometer (Tokyo, Japan) was used for fluorimetric measurements. The slit width of both excitation and emission monochromator was set at 3 nm. Infrared spectrophotometer, Shimadzu, was used for recording IR spectra. NMR-spectrophotometer (480 MHz) EM390 NMR Spectrometer was used for recording ¹H-NMR spectra ¹H- in DMSO-d₆ at 480 MHz.

Pharmaceuticals

OMZ and RAB (Sigma, Quisna, El-Menoufia, Egypt), LAN is kindly supplied by NODCAR, Cairo, Egypt. PAN (Uni Pharma, Cairo, Egypt). All were checked for their purity (98.89± 0.56- 99.65± 0.78) by pharmacopoeial methods [2,3].

Pharmaceutical formulation

- Omekap capsules® (Sedico, 6th October city, Egypt), each is labeled to contain 20 mg OMZ.
- Losec tablets® (Astrazeneca, 6th October city, Egypt), each is labeled to contain 20 mg OMZ magnesium equivalent to 20 mg OMZ.
- Risek vials® (Julphar, Ras Al Khaimah, U.A.E), each is labeled to contain 40 mg OMZ sodium equivalent to 40 mg OMZ.
- Zollipak capsules® (Sedico, 6th October city, Egypt), each is labeled to contain 15 mg LAN.
- Pantoloc tablets® (MUP, Cairo, Egypt), each is labeled to contain 20 mg PAN.
- Pantazol vials® (Sigma, Quisna, El-Menoufia, Egypt), each is labeled to contain 40 mg PAN.
- Rabacid tablets® (Sigma, Quisna, El-Menoufia, Egypt), each labeled to contain 10 mg RAB.

Reagents and their solutions

- Sodium hydroxide (El Nasr Pharmaceutical Chemicals co., Egypt).
Sodium hydroxide solution: 0.2 N NaOH in double distilled water.
- Iodine (El Nasr Pharmaceutical Chemicals co., Egypt).
Iodine solution: 0.4 g % w/v dissolved in methanol.
- Sodium 1, 2-naphthoquinone-4- sulphonate (Acros, Ceel, Belgium).
Sodium 1, 2- naphthoquinone-4- sulphonate solution: 0.02g % w/v dissolved in 0.2 N NaOH.
- Methanol of analytical grade (El Nasr Pharmaceutical Chemicals co., Egypt).
- Double distilled water.

All solvents and other chemicals used throughout this study were of analytical grade.

Preparation of Standard solution

An accurately weighed amount (50 mg) of OMZ, LAN, PAN or RAB was transferred into a 100-mL calibrated flask, and dissolved in about 10-mL methanol. The solution was completed to the mark with double distilled water to provide a stock solution containing 0.5 mg mL⁻¹ of each drug. The working standard solutions were prepared by further dilution of the suitable aliquots of the stock solution with double distilled water to obtain a range of 50-600, 100-800, 100-650 and 100-850 ng mL⁻¹ for OMZ, LAN, PAN and RAB, respectively.

Preparation of sample solutions

Tablets and capsules

The contents of twenty tablets or capsules were accurately weighed, finely powdered and mixed thoroughly. The average weight of one tablet or one capsule was then calculated. An accurately weighed quantity of powder equivalent to 50 mg of OMZ, PAN or RAB was quantitatively to a 100- mL calibrated flask. About 50 mL of methanol were added to the content of the flask, shaken well for 5 minutes and sonicated for further 5 minutes. The volume was made up with double distilled water, mixed well, filtered, and the first portion of filtrate was discarded. The obtained filtrate was further diluted so as to obtain a stock solution of 500 ng mL⁻¹.

Vials

An accurately weighed amount of the powder equivalent to 50 mg of OMZ or PAN was then transferred to a 100- mL calibrated flask. About 50 mL of methanol were added to the content of the flask, shaken well for 5 minutes and sonicated for further 5 minutes. The procedure was then completed as under Tablets and capsules.

General procedure

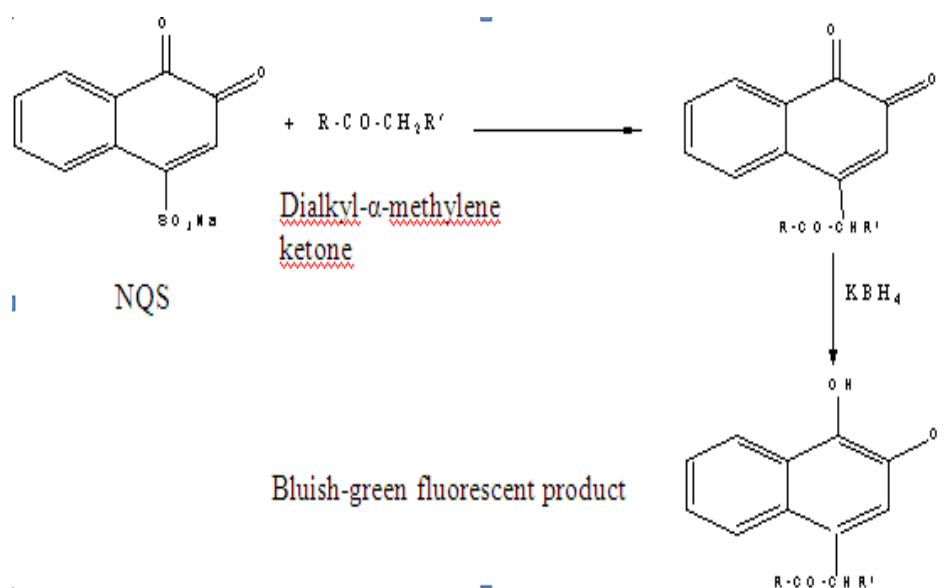
One milliliter of each of the working standards or sample solutions, of OMZ, LAN, PAN or RAB was transferred to 10- mL calibrated flask. A volume of 0.8 mL of NQS reagent was added to the content of the flask followed by one milliliter of methanolic solution of iodine and mixed well. The solution was heated in a thermostatic water bath adjusted at 70°C for 30 minutes and then, prechilled in ice-bath for 5 minutes. The volume was made up to 10-mL with double distilled water. RFI of the resulting solutions were measured at $\lambda_{exc.} = 340$ nm, $\lambda_{em.} = 480$ nm, against blank experiments treated similarly.

RESULTS AND DISCUSSIONS

Replacement of the sulphonate group of naphthoquinone- sulphonic acid by the active methylene compound and reduction to the corresponding dihydroxynaphthalene having bluish- green fluorescence was previously reported Scheme 1 [40]. Based on this reaction, preliminary experiments were performed in this work in order to study similar reactions of active methylene group of the studied PPIs with NQS. However, results proved that no fluorescence was obtained even after reduction with potassium borohydride. This could be due to the steric hindrance and the existence of sulphonate group that shifts the reaction backward. So, oxidation of sulphonate group was urgently needed to aid the process of desulphonation of NQS and to facilitate the coupling process. Therefore, several oxidizing agents as: iodine, hydrogen peroxide, ceric sulphate, potassium permanganate, potassium dichromate and potassium persulphate were tested. Best results were observed upon using iodine and heating without the need for reduction with potassium borohydride. However, a weak fluorescence was obtained upon reduction with borohydride. On the other hand, oxidation with hydrogen peroxide resulted in a weak fluorescence intensity while no fluorescence was observed with other tested oxidizing agents as shown in Table 1.

Table 1: Effect of some oxidizing agents on RFI of the reaction product of OMZ with NQS

Oxidant/reductant	Excitation		Emission	
	λ (nm)	RFI	λ (nm)	RFI
Iodine on cold	380	20.12	420	37.12
Iodine with heating	340	50	480	80.2
Hydrogen peroxide (20, v/v) on cold	380	23	430	54.34
Iodine with heating and addition of borohydride	380	15	450	45
Iodine with heating and addition of borohydride and HCl	340	23.89	440	30.23



Scheme 1: Reported reaction mechanism between NQS and active methylene containing compounds.

Spectral characteristic

The proposed spectrofluorometric method is based on the reaction of the studied PPIs with NQS in presence of iodine solution. Fig. 2 shows the excitation and emission spectra of the fluorescent reaction product at λ_{exc} (340 nm) and λ_{em} . (480 nm).

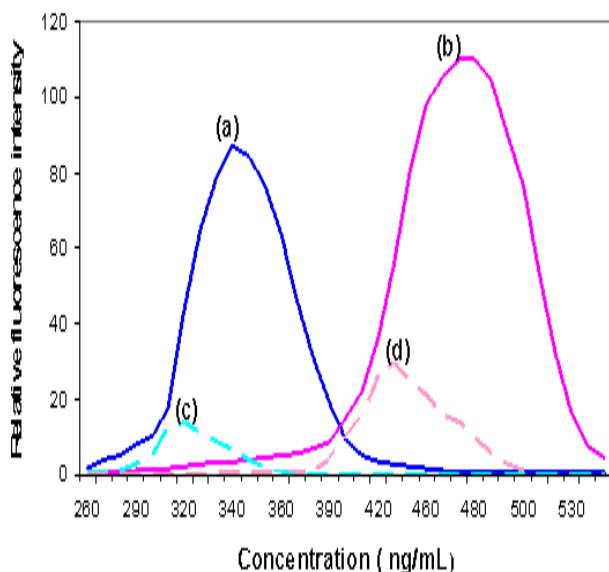


Fig. 2: Excitation (a) and emission (b) spectra of OMZ reaction product with NQS and blank (c,d) treated similarly. Drug concentration was 50 ng mL^{-1}

Optimization of variables

Results proved that relative fluorescence intensity of the reaction product of PPIs increased by using 0.8 ml of 0.02 g % w/v NQS and 1.0 mL of 0.4 g % w/v of iodine reagent. The influence of applying different heating temperatures and incubation times in a thermostatically controlled water bath was studied. Optimum temperature was found to be $70.0 \pm 2.0^\circ\text{C}$. Complete reaction was attained in a period of 30 minutes for all studied PPIs. Results proved that by using solvents of different polarities positions of the excitation and emission maxima as well as the Stokes shifts (λ_{exc} -

λ_{em}) were only slightly affected, However the relative fluorescence intensity is greatly affected. Maximum relative fluorescence intensity was obtained when water is used as diluting solvent. This could be attributed to the high solubility of the formed product in water. It was found that the formed fluorescent product remained stable for further 35 minutes at room temperature ($25 \pm 1^\circ\text{C}$) after dilution with water. Then, a gradual decrease in the RFI was observed. Therefore, all measurements in the whole study were recorded within 30 min.

Validation of the proposed spectrofluorometric method [41].

Linearity, detection and quantitation limit

Under the proposed experimental conditions, the relationship between the relative fluorescence intensity and concentration for each of the studied drug was quite linear in the concentration range 5-60, 10-80, 10-65 and 10-85 ng mL^{-1} for OMZ, LAN, PAN and RAB, respectively. The regression equations were derived using the least square method. The intercepts (a), slopes (b), correlation coefficients (r), limit of detection (LOD), and limit of quantitation (LOQ), for all the studied drugs are summarized in the Table 2.

Accuracy

was checked by applying the proposed spectrofluorometric method for the assay of the studied PPIs in their corresponding pharmaceutical dosage forms. Table 3 shows good percent recoveries (98.5-100.6 %, 1.4-1.1).

Precision

The precision of the proposed spectrofluorometric method was checked by replicate analysis of five separate solutions of the working standard of each of the studied proton pump inhibitors, at three concentration levels. The relative standard deviations were less than 2% in all cases, indicating good repeatability of the proposed method Table 4. Inter-day and intra-day precision were also studied with good recoveries, indicating high precision.

Robustness

Robustness [42] of the method was examined by evaluating the influence of small variations in the variables of the proposed method including: concentration and volume of NQS reagent, concentration and volume of iodine, temperature, and heating time on the method suitability and sensitivity. Results proved that none of these variables significantly affect the relative fluorescence intensity, Table 5. This indicates the reliability of the method during normal usage and so it can be considered robust.

Table 2: Quantitative parameters for the studied PPIs using the proposed spectrofluorometric method

Authentic drug	Linearity range (ng mL ⁻¹)	Correlation coefficient (r) ± SD*	Determination coefficient (r ²) ± SD*	Intercept ± SD*	Slope ± SD*	Regression equation	LOD** (ng mL ⁻¹)	LOQ• (ng mL ⁻¹)
OMZ	5-60	0.9993± 2.7×10 ⁻⁴	0.9986± 7.3×10 ⁻⁸	-2.415± 0.461	0.986± 0.033	Y= - 2.415+0.986X	1.54	4.67
LAN	10-80	0.9996± 2.8×10 ⁻⁴	0.9991± 7.8×10 ⁻⁸	-2.799± 0.438	0.565± 0.0159	Y= - 2.799+0.565X	2.56	7.75
PAN	10-65	0.9996± 4.6×10 ⁻⁴	0.9991± 2.2×10 ⁻⁷	-3.205± 0.786	0.876± 0.043	Y= - 3.205+0.876X	2.96	8.97
RAB	10-85	0.9992± 2.8 ×10 ⁻⁴	0.9984± 7.8×10 ⁻⁸	-4.210± 0.730	0.805± 0.018	Y= - 4.210+0.805X	2.99	9.07

* Results are calculated based on standard curves. ** Standard deviation of five replicates. • Coefficient of variation.

Table 3: Accuracy of the proposed spectrofluorometric method at three concentration levels

Exp. No.	% Recovery											
	OMZ*			LAN*			PAN*			RAB*		
	10	20	40	10	20	40	10	20	40	10	20	40
1	98.4	99.5	98.4	101.4	98.1	99.3	99.1	97.4	99.7	97.9	98.7	99.5
2	97.8	100.7	100.9	101.2	97.3	102.2	101.4	99.6	97.9	97.9	101.4	98.5
3	98.9	101.2	101.3	99.5	100.5	100.6	100.4	99.9	98.7	99.9	99.2	99.3
4	99.8	101.2	100.5	98.1	99.4	99.8	98.8	99.2	98.7	98.1	97.9	99.6
5	99.1	100.6	100.2	99.3	100.2	98.6	99.3	97.2	98.9	99.9	100.3	97.4
Mean ±	98.8±	100.6±	98.8±	99.9±	99.1±	100.1±	99.8±	98.5±	98.8±	98.7±	99.5±	98.9±
SD**	0.75	0.69	1.12	1.39	1.37	1.27	1.08	1.42	0.64	1.06	1.37	0.87
C.V•	0.76	0.69	1.12	1.39	1.38	1.27	1.08	1.44	0.65	1.07	1.38	0.88

* Results are calculated based on standard curves. ** Standard deviation of five replicates. • Coefficient of variation.

Table 4: Inter-day and intra-day precision of the proposed spectrofluorometric method at three concentration levels.

Authentic drug	Concentration (ng mL ⁻¹)	Intraday precision		Interday precision	
		% Recovery ± SD*	C.V**	% Recovery ± SD*	C.V**
OMZ	10	98.4 ± 0.55	0.56	99.3 ± 0.47	0.47
	20	100.5 ± 0.87	0.87	101.0 ± 0.35	0.35
	40	100.2 ± 1.47	1.47	100.7 ± 0.57	0.57
LAN	10	100.7 ± 1.04	1.03	98.9 ± 0.76	0.77
	20	98.6 ± 1.47	1.49	100.0 ± 0.57	0.57
	40	100.7 ± 1.45	1.44	99.7 ± 1.01	1.01
PAN	10	100.3 ± 1.15	1.15	99.5 ± 0.82	0.82
	20	98.9 ± 1.37	1.39	98.8 ± 1.40	1.42
	40	98.8 ± 0.90	0.91	98.8 ± 0.12	0.12
RAB	10	98.6 ± 1.15	1.17	99.3 ± 1.04	1.05
	20	99.8 ± 1.44	1.44	99.1 ± 1.20	1.21
	40	99.1 ± 0.53	0.53	98.8 ± 1.19	1.20

* Standard deviation of five replicates. ** Coefficient of variation.

Application to pharmaceutical dosage forms

The proposed spectrofluorometric method was applied to the determination of PPIs in tablets, capsules and vials Table 6. Results are compared with a reported visible spectrophotometric method [43, 44]. Results of the proposed spectrofluorometric method were found comparable with those of reported methods as indicated in t- and F- tests. Also, recovery studies were performed by using standard addition method [45].

This depends upon the addition of a known quantity of the standard PPIs to the corresponding pharmaceutical sample PPIs, and then the resulting solution was analyzed by the proposed spectrofluorimetric method. Results presented in Table 7 indicate good recoveries (96.9-102.7 % ± 0.1- 0.1) and confirm the absence of interference due to common excipients and hence, accuracy of the proposed method.

Investigation of the reaction mechanism

Reaction mechanism of PPIs with NQS in alkaline medium was investigated using OMZ as a model compound of the studied drugs. It

was found that the melting point of the product was 280° C. The isolated product gave only one single spot on TLC plate that confirms formation of only one compound.

UV-VIS spectra

The absorption spectrum of the methanolic solution of OMZ (λ_{max} = 308 nm) was found to be different from that of the isolated product (λ_{max} = 340 nm), Fig. 3.

Job's plot of continuous variation

Job's method of continuous variation [193] revealed that the maximum RFI was obtained when the molar ratio of the measured product is 1:1 (drug: NQS), Fig. 4.

IR spectra

The IR spectrum [46] obtained from the isolated reaction product was compared with pure OMZ. It was evidence from the obtained spectra that disappearance of CH₂ out-of-plane bending of OMZ (882 cm⁻¹) refers to the reaction of this group, this band usually appears between 675-900 Cm⁻¹. Also, presence of a signal of -C=O at 1610

cm⁻¹ is indicative for introduction of this group into OMZ through the coupling with NQS. Furthermore, appearance of signal for the

following groups from NQS in the product: - Signals at 1011 cm⁻¹, 1196 cm⁻¹, 815 cm⁻¹ and at 1069 cm⁻¹, Fig. 5.

Table 5: Robustness of the proposed spectrofluorometric method

Variation	% Recovery \pm SD*			
	OMZ*	LAN*	PAN*	RAB*
** No variation	100 \pm 0.3	99.1 \pm 0.2	98.8 \pm 0.6	99.3 \pm 0.4
NQS conc. \pm 0.005%				
0.015	97.1 \pm 0.5	97.2 \pm 0.7	97.8 \pm 1.2	97.7 \pm 1.3
0.025	98.4 \pm 0.5	97.7 \pm 0.6	97.9 \pm 0.9	98.5 \pm 1.1
NQS volume \pm 0.05 ml				
0.75	97.4 \pm 1.1	97.2 \pm 0.4	97.4 \pm 0.6	97.8 \pm 1.1
0.85	99.1 \pm 0.5	97.2 \pm 0.5	98.8 \pm 0.7	98.9 \pm 0.8
Iodine conc. \pm 0.02%				
0.38	98.8 \pm 0.5	97.9 \pm 0.4	97.9 \pm 0.5	98.1 \pm 0.5
0.42	99.1 \pm 0.3	97.2 \pm 0.5	97.7 \pm 0.9	99.6 \pm 0.8
Iodine volume \pm 0.05 ml				
0.95	98.6 \pm 0.5	97.6 \pm 0.7	99.5 \pm 0.4	98.3 \pm 0.8
1.05	99.1 \pm 0.5	98.2 \pm 0.3	98.9 \pm 0.5	98.3 \pm 0.2
Heating temperature \pm 3° C				
67	98.5 \pm 0.6	98.9 \pm 0.6	98.3 \pm 0.5	97.6 \pm 1.1
73	99.4 \pm 0.8	98.7 \pm 0.6	97.6 \pm 0.6	97.1 \pm 0.7
Heating time \pm 2 min.				
28	99.3 \pm 0.2	97.0 \pm 0.8	97.9 \pm 0.2	97.8 \pm 1.1
32	99.4 \pm 0.2	97.7 \pm 0.6	101.7 \pm 0.6	102.7 \pm 0.7

Mean of five determinations. the proposed method. ** No variation in the assay conditions of • Drug concentration was 50 ng ml⁻¹.

Table 6: Assay of the studied PPIs in their pharmaceutical dosage forms by proposed spectrofluorometric method

Authentic drug	Pharmaceutical formulation	% Recovery \pm SD ^a		t-value	F-value
		Proposed	Reported		
OMZ*	Omeprazole® (capsule)	101.8 \pm 0.52	98.2 \pm 0.35	0.453	1.004
	Risek® (vial)	98.6 \pm 0.62	99.0 \pm 0.46	0.761	1.762
	Losec® (tablet)	102.5 \pm 0.31	99.9 \pm 0.51	0.836	0.570
LAN**	Zollipak® (capsule)	100.1 \pm 0.8	100.1 \pm 0.51	0.267	4.245
PAN*	Pantoloc® (tablet)	101.5 \pm 0.5	101.7 \pm 0.24	0.234	0.512
	Pantazol® (vial)	99.1 \pm 0.3	99.8 \pm 0.78	0.419	0.254
RAB*	Rabacid® (tablet)	100.8 \pm 0.3	99.3 \pm 0.23	0.629	1.412

^a Standard deviation of five replicates. * Reference [43]. ** Reference [44]. Theoretical values at 95 % confidence level: t = 2.306, F= 6.388

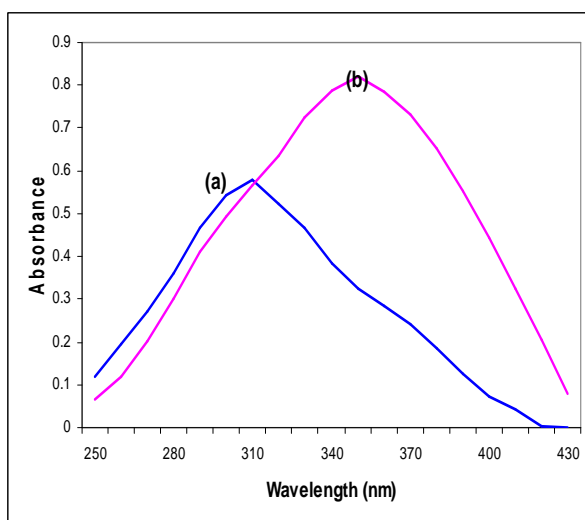


Fig. 3:- Job's plot of continuous variation of OMZ with NQS.

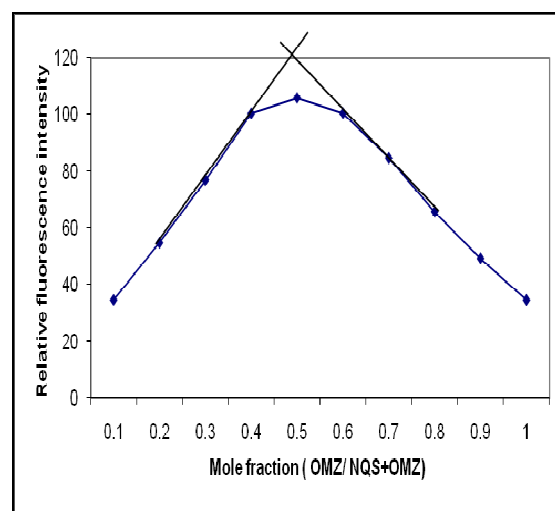


Fig. 4: UV-VIS spectra of omeprazole (a) and isolated fluorescent product (b).

Table 7: Standard addition method for the assay of the studied PPIs in their pharmaceutical dosage forms by the proposed method.

Authentic drug	Pharmaceutical formulation	Authentic drug Added (mg)	Authentic drug found (mg)	Recovery (%)± SD*
OMZ	Omeprazole® capsules	5	4.97	99.4±0.2
		10	9.80	98.0±0.1
		15	15.25	101.6±0.4
		20	20.35	101.7±0.1
	Losec® tablets	5	4.94	98.8±0.2
		10	10.30	103.0±0.2
		15	15.15	101.0±0.2
		20	20.19	100.9±0.1
	Risek® vials	5	4.94	98.8±0.1
		10	9.89	98.9±0.1
		15	14.86	99.1±0.5
		20	19.94	99.7±0.5
LAN	Zollipak® capsules	5	5.05	101.0±0.1
		10	10.20	102.0±0.2
		15	15.40	102.7±0.1
PAN	Pantoloc® tablet	5	4.97	99.5±0.1
		10	9.89	98.9±0.1
		15	14.91	99.4±0.1
	Pantazole® vials	20	19.54	97.7±0.5
		5	4.93	98.6±0.2
		10	9.69	96.9±0.1
		15	14.69	97.9±0.5
RAB	Rabacid® tablets	20	19.73	98.6±0.3
		5	4.88	97.6±0.2
		10	9.72	97.2±0.1
		15	14.79	98.6±0.1
		20	19.82	99.1±0.2

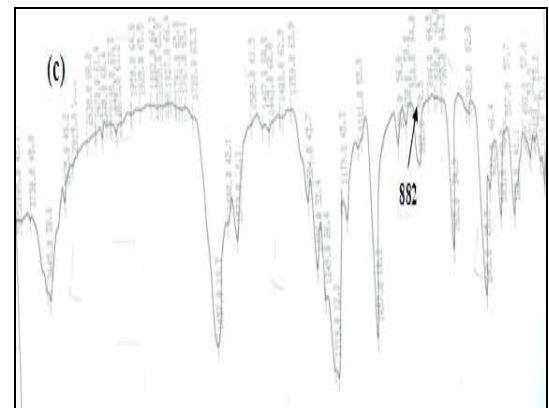
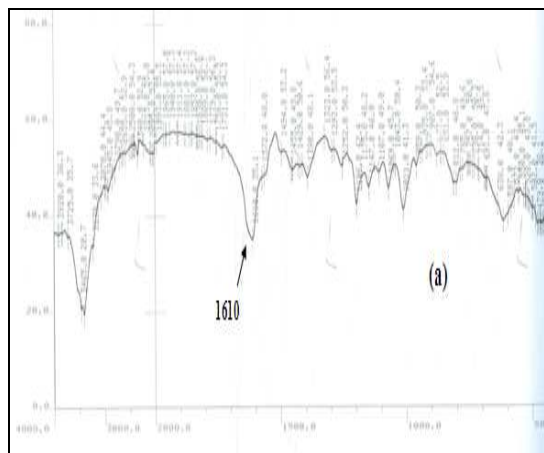
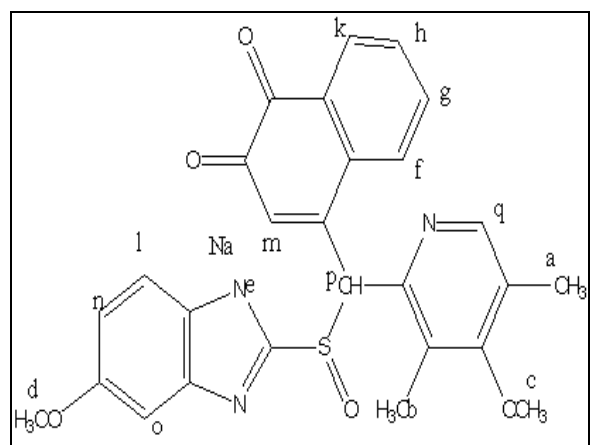
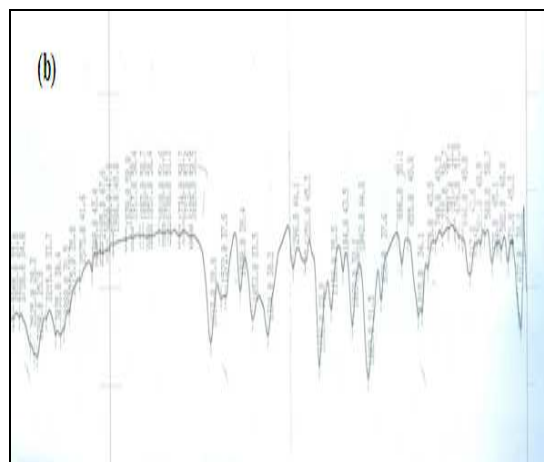


Fig. 5: IR-spectra of the isolated reaction product (a), NQS (b) and OMZ (c).



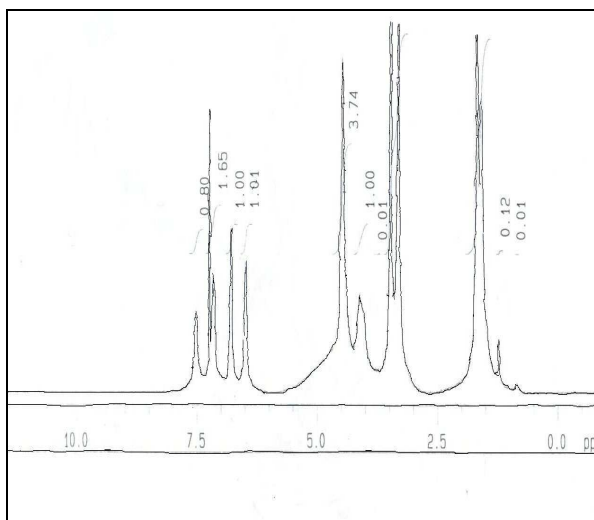


Fig. 6: NMR-spectrum of the isolated fluorescent reaction product of OMZ and NQS.

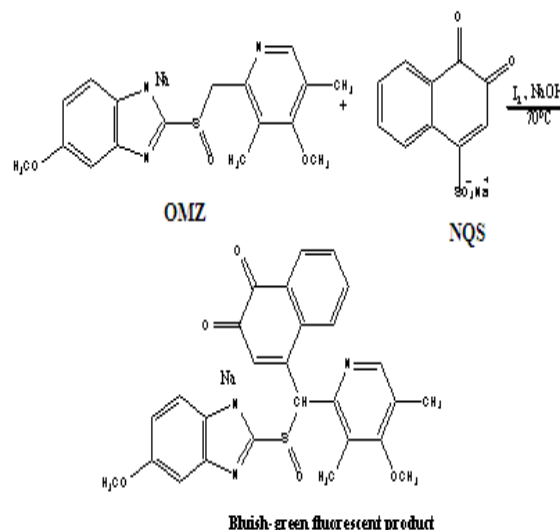


Table 8: Chemical shifts (δ -values, ppm) of spectrofluorometric product.

Chemical shift (ppm), δ -values	Number of protons (multiplicity)	Assignment
3.50	3(s)	a-CH ₃
3.50	3(s)	b-CH ₃
3.80	3(s)	c-OCH ₃
3.80	3(s)	d-OCH ₃
4.20	1(s)	e-NH
4.50	4(s)	f,g,h,k,l-CH
6.50	1(s)	m-CH
6.80	1(s)	p-CH
7.20	2(s)	n,o-CH
7.55	1(s)	q-CH

NMR spectra

NMR- spectrum of the isolated product in deuterated chloroform containing tetramethylsilane as internal standard was recorded. Table (8) shows the chemical shifts (δ -values, ppm) multiplicity and the integration obtained for each kind of proton in the product. Signals observed at δ , 2.50 is characteristic band for TMS and that observed at δ ,7.4 is also characteristic for the water present in the solvent [46], Fig. 6.

Reaction mechanism

From the above mentioned spectral data, we can suggest the reaction mechanism with high degree of certainty to be as in scheme [2].

CONCLUSION

A rapid, sensitive and validated spectrofluorometric method has been developed for the quantitation of some PPIs based on coupling with sodium 1,2-naphthoquinone-4-sulphonate (NQS) in presence of methanolic solution of iodine and alkaline medium. The present method employs mild condition, simple, inexpensive and readily available chemicals and instrument. Mechanism of reaction was suggested based on IR and NMR spectra. Thus, it can be concluded that due to simplicity, reliability and cost of analysis.

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