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RESEARCH ARTICLE

A Validated Spectrophotometric assay of some proton pump inhibitors using diazotized p-nitroaniline in alkaline medium

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ABSTRACT

Simple, rapid and economic Spectrophotometric method was developped for the assay of certain proton pump inhibitors (PPIs) belonging to benzimidazole derivatives namely; omeprazole, rabeprazole, pantoprazole and lansoprazole. The method depends on the reaction of the cited proton pump inhibitors with diazotized p-nitroaniline in alkaline medium using 1.5 N NaOH and dimethylformamide. The produced green color was measured at 590 nm for omeprazole, rabeprazole and 610 nm (for, pantoprazole and lansoprazole). The recorded absorbances obey Beer's law for 5-30, 10-35, 5-40 and 5-40 µg ml^{-1} omeprazole, rabeprazole, pantoprazole and lansoprazole, respectively with good correlation coefficients (0.9996-0.9999). The limits of detection and quantification are also reported for the studied method. Intra-day and inter-day precision as well as accuracy and robustness of the method have been evaluated. The proposed method was successfully applied to the assay of the studied proton pump inhibitors in pharmaceutical dosage forms and the results were statistically compared with those of reported method by applying Student's t-test and F-test where no significance differences were observed.

Keywords: omeprazole, rabeprazole, pantoprazole, lansoprazole, spectrophotometry, p-nitroaniline, pharmaceuticals analysis.

1. INTRODUCTION:

Omeprazole (OMP), lansoprazole (LAN), pantoprazole (PAN), lansoprazole (LAN), and rabeprazole (RAB) (Fig. 1) are members of benzimidazole class of drugs. They are important benzimidazole derivatives which are used in the treatment of gastric and duodenal ulcers, and reflux oesophagitis [1]. Their efficacy as antiulcer and anti-secretory agents has been well established. A literature survey reveals that several methods have been used for determination of the above mentioned drugs in pharmaceutical dosage forms and biological fluids alone or in combination with other drugs including titrimetry [2], [3] UV-spectrophotometry, [4-11], Colorimetry, [12-17] spectrofluorimetric [5] , [18],[19] , high performance thin-layer chromatography [20-29] , high performance liquid chromatography [22], [23], [30 - 37] . Capillary electrophoresis [7] and electrochemical methods [38- 39]. The ultraviolet-visible spectrophotometric methods are still considered the most simple, convenient and economic technique for routine analysis of drugs in pure and pharmaceutical dosage forms compared to other methods [30-39] Therefore, the present work aimed at studying the azodye formation of the studied PPIs namely; OMZ, LAN, PAN and RAB with diazotized p-nitroaniline in alkaline medium and measuring the product at 590 nm (for OMZ, RAB) or 610 nm (for PAN, LAN).

2-EXPERIMENTAL:

2.1-Apparatus. A Shimadzu model UV-1700 PC (Tokyo, Japan) UV-VIS double beam spectrophotometer with matched 1-cm quartz cells was used for recording the electronic absorption spectra and all measurements. 1H-NMR spectra of the isolated reaction product were recorded in DMSO-d6 at 480 MHz by JNM-LA 480 FT NMR system, (Jeol, oxford). IR spectra were recorded by IR -470, (Japan).

2.2-Pharmaceuticals

OMZ and RAB are kindly supplied by Sigma, Quisna, El-Menoufia, Egypt; LAN is supplied by NODCAR, Cairo, Egypt, while PAN is supplied by Uni Pharma, Cairo, Egypt.

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All were checked for their purity (98.89± 0.56- 99.65± 0.78) by pharmacopoeial methods [2], [3].

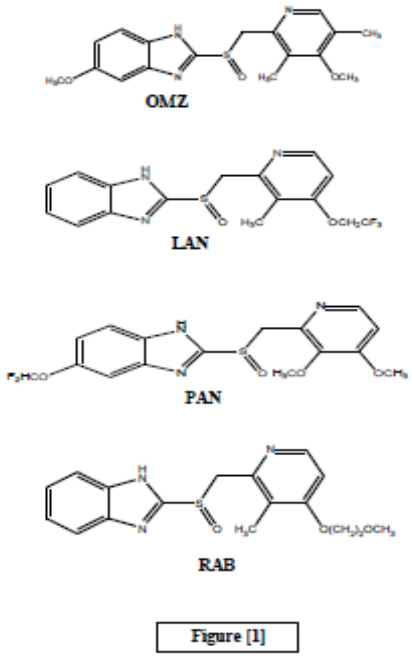


Figure [1]

Figure [1]:- Chemical structures of the studied proton pump inhibitors.

2.3-Pharmaceutical formulation

Omepak 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 OMZ magnesium equivalent to 20 mg OMZ. Risek vials® (Julphar, Ras Al khaimah, U.A.E), each is labeled to contain 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. Rabicid tablets® (Sigma, Quisna, El-Menoufia, Egypt), each labeled to contain 10 mg RAB.

2.4- Reagents and their solutions

• Sodium hydroxide (El Nasr Pharmaceutical chemicals co., Egypt).

- Sodium hydroxide solution: - 0.2 N and 1.5 N NaOH in double distilled water.

• P-nitroaniline (Sigma-aldrich co. Ltd., Steinheim, Germany).

- P-nitroaniline solution: A weighed amount of p-nitroaniline (0.8 g) was transferred into 250 mL beaker and dissolved in 25 ml hydrochloric acid. The contents of the beaker were quantitatively transferred into a 250 ml calibrated flask, completed to volume with double distilled water and mixed well.

• Sodium nitrite (BDH, Poole, UK).

- One gram of sodium nitrite was transferred to a 250 ml flask, completed to volume with double distilled water and mixed well.

• Dimethylformamide (Scharlau Chemie S.A., Barcelona, Spain).

• Hydrochloric acid (El Nasr Pharmaceutical chemicals Co., Egypt).

• Diazotized p-nitroaniline solution: A volume of 10 ml p-nitroaniline solution was transferred into a 250 ml beaker. Then, 10 ml of sodium nitrite solution was added and mixed well. The prepared diazonium salt is stable at room temperature for about six hours.

• Double distilled water, methanol (BDH, Poole, UK), and dioxan (Ranbaxy, New-Delhi)

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

2.5-Preparation of Standard and Sample Solutions.

2.5.1- Preparation of Standard Solutions.

Into a 100 ml calibrated flask, 200 mg of OMZ, LAN,RAB or PAN was accurately weighed, dissolved in 20 ml 0.2 N NaOH and completed to volume with the same solvent to obtain a stock solution of 2 mg ml^{-1}. These stock solutions were further diluted with 0.2 N NaOH to obtain suitable concentrations in the ranges from 50-300, 100-350, 50-400, 50-400 µg ml^{-1} for OMZ, LAN, PAN and RAB, respectively.

2.5.2- Preparation of tablets or capsules sample solutions. Ten tablets or the contents of ten capsules of each formulation were weighed and finely powdered. A quantity of the powder equivalent to 200 mg of the drug was transferred into a 100 ml calibrated flask, dissolved in 20 ml of 0.2N NaOH, swirled and sonicated for 5 minutes. The flask was completed to volume with the same solvent, shaken well for 10 minutes, and filtered. The first portion of the filtrate was rejected and a measured volume of the filtrate was diluted quantitatively with the respective solvent to yield suitable concentrations in the linear range of each particular assay method.

2.5.3- Preparation of vials sample solutions. An accurately weighed amount of the powder equivalent to 20 mg of OMZ or PAN was then transferred into a 100-ml calibrated flask. About 50 ml of 0.2 N NaOH solution 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 0.2 N NaOH solution, mixed well, filtered, and the first portion of filtrate was discarded. The obtained filtrate was then used as a stock sample solution.

2.6- General Analytical Procedure. One milliliter of the working standard solution or sample solution of OMZ, LAN, PAN or RAB was transferred into 10 ml calibrated flask. A volume of 1.5 ml of the freshly prepared diazonium salt was added and the solution was allowed to

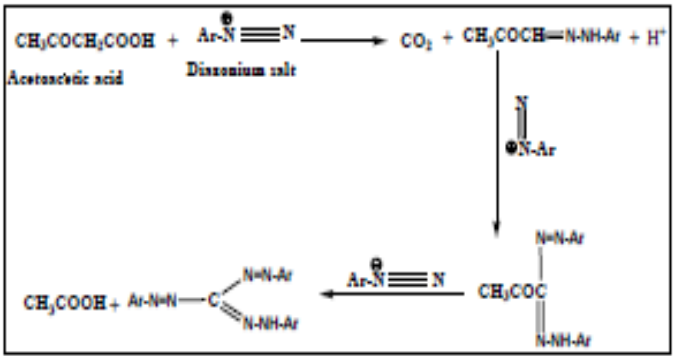
stand for six minutes. Then, 0.8 ml of 1.5 N NaOH was added, followed by 2 ml of DMF. The solution was diluted to 10 ml with the suitable solvents (dioxan for OMZ and RAB, methanol for PAN and LAN). The colored product was measured at λ_{max}=590 nm (for OMZ, RAB) or 610 nm (for LAN, PAN) against a blank experiment treated similarly. Concentrations are calculated from standard curves or regression equations.

2.7. Preparation and extraction of the isolated product for spectral analysis

An accurately weighed amount of 150 mg pure PAN was transferred to a beaker and dissolved in 20 ml 0.2 N NaOH. Five milliliters of the prepared diazonium salt was added followed by 4 ml of 1.5 N NaOH and 6 ml DMF. Reaction was allowed to stand for 6 minutes and finally diluted with 10 ml dioxan. The product was shaken with chloroform where it was extracted to the aqueous layer but other impurities were extracted to the organic layer. Then, the aqueous layer was evaporated using rotary evaporator. TLC was used to check purity of the product.

3- Results and discussions.

The interaction of diazonium salts with active methylene compounds as acetoacetic acid forming highly colored species was previously reported [40, 41] scheme (1).



Scheme [1]: Reported reaction mechanism betweendiazonium salt and active methylene containing compounds

Based on this reaction, we proposed a spectrophotometric method depending on reaction of active methylene group of the studied PPIs with diazotized p-nitroaniline in alkaline medium to yield colored products which are measured at λ_{max} = 590 nm (for OMZ, RAB) or 610 nm (for LAN, PAN). Many reagents as p-aminobenzoic acid, sulphanilic acid, 2-aminobenzothiazole, aniline, and p-nitroaniline were preliminary tested for preparation of diazonium salt then coupling with the studied PPIs. However, p-nitroaniline was found to be the best reagent for this coupling reaction. Although, p-nitroaniline was more soluble in methanol than in hydrochloric acid but the precision was improved by the latter. Moreover, hydrochloric acid was found to be the best acid for diazotization.

3.1- Spectral characteristics. The azodye interaction product of the studied PPIs with diazotized p-nitroaniline in alkaline medium shows absorption maxima at λ_{max}= 590 nm (for OMZ, RAB) or 610 nm (for LAN, PAN), Fig. (2)

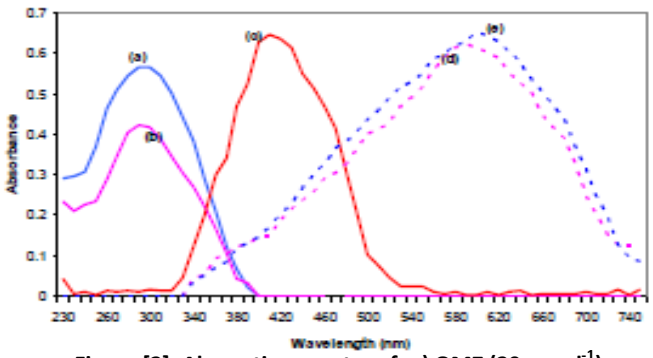


Figure [2]: Absorption spectra of: a) OMZ (20 µg ml^{-1}). b) PAN (20 µg ml^{-1}). c) Diazotized p-nitroaniline. d) Reaction product of OMZ. e) Reaction product of PAN.

3.2-Optimization of reaction conditions

3.2.1-Optimization of diazonium salt preparation

Results showed that absorbances were increased by increasing the concentration of sodium nitrite up to 0.4 g % w/v and p-nitroaniline reagent up to 0.32 g % w/v. Also, it was found that 25 mL of HCl are optimum for dissolution of reagent in 250 ml of solution.

3.2.2- Optimization of azodye formation.

Maximum absorption intensity was obtained by increasing the volume of diazonium salt till optimum value which is 1.5 mL. and by using 0.8 ml of 1.5 N NaOH. However, the intensity of azodye product was highly improved in the presence of organic base. This may be probably due to increasing liability of the hydrogen of active methylene group. Therefore, several organic bases have been tested beside NaOH including: dimethylformamide, aniline, dimethylaniline and pyridine. It was found that 2 ml of dimethylformamide was the best solvent for optimum intensity of the chromogen (Table 1).

Authentic drug	OMZ	LAN	PAN	RAB
Linearity range (µg mL^{-1})	5-30	10-35	5-40	5-40
Correlation coefficient (r)± SD*	0.9998 ± 9.8×10^{-5}	0.9997 ±2.8×10^{-4}	0.9997± 3.3×10^{-4}	0.9996±3.5×10^{-4}
Determination coefficient (r^2) ± SD*	0.9995 ±9.5×10^{-9}	0.9994 ± 7.8×10^{-8}	0.9994 ± 1.1×10^{-7}	0.9992 ±1.2×10^{-7}
Intercept (a) ± SD*	0.07±7.40×10^{-3}	-0.12 ±1.38×10^{-3}	0.11± 2.08×10^{-2}	0.095 ± 1.30×10^{-3}
Slope (b)± SD*	0.029±1.2×10^{-4}	0.023± 2.1×10^{-4}	0.024± 5.3×10^{-4}	0.023±8.2×10^{-4}
Regression equation	Y=0.07+0.029X	Y= -0.12 +0.023X	Y=0.11+0.024 X	Y=0.095+0.023X
LOD[a] (µg mL^{-1})	0.84	0.19	2.86	0.19
LOQ[b](µg mL^{-1})	2.55	0.61	8.67	0.57

* Average of five replicates.
[a] Limit of detection.
[b] Limit of quantitation.

Table [1]: Quantitative parameters for the studied PPIs using the proposed spectrophotometric method.

Complete reaction was attained in a period of six minutes for all studied PPIs. Maximum absorbance was obtained when methanol is used as diluting solvent for LAN and PAN, and dioxan as a diluting solvent for OMZ and RAB. It was found that after dilution with the suitable solvent, the formed product remained stable for further 90 minutes at room temperature (25 ±1°C). Then gradual decrease in the absorbance was observed due to breaking of the azo group double bond in alkaline medium

3.3- Validation of the proposed method. [42]

3.3.1- Linearity, detection limit and quantitation limit. Validation was performed according to ICH guidelines [42]. The linearity of the produced coloured product was investigated in the range of 5-30, 10-35, 5-40 and 5-40 µg ml^{-1} for OMZ, LAN, PAN and RAB, respectively. Correlation coefficients ranged from 0.9996 to 0.9998 which indicates that the proposed method is suitable for the quantitative analysis of the studied proton pump inhibitors. It is obvious from the values of LOD and LOQ for all the studied PPIs prove that the proposed method is sensitive to measure the studied PPIs in their pharmaceutical formulations; Table (2) presents quantitative parameters of the proposed method.

EXP. No.	OMZ			LAN			PAN			RAB		
	10	20	30	10	20	30	10	20	30	10	20	30
1	98.2	98.8	99.6	100.3	99.8	99.6	100.7	98.7	98.1	98.4	100.8	100.2
2	98.2	99.6	100.4	100.3	99.6	100.5	102.2	100.0	100.1	99.1	99.2	99.6
3	99.0	100.7	100.1	101.2	99.2	99.2	99.8	100.4	99.1	99.8	99.7	99.1
4	99.0	100.9	100.1	100.4	98.8	99.5	101.0	99.1	99.1	101.6	100.3	99.6
5	101.6	99.8	99.5	101.1	99.6	99.8	99.9	98.7	100.0	102.6	100.7	99.8
6	101.2	99.8	100.1	99.6	99.9	100.0	99.0	100.2	99.7	100.0	101.8	100.2
Mean	99.5 ±	99.9 ±	98.8 ±	100.5 ±	99.5 ±	99.8 ±	100.4 ±	99.5 ±	99.4 ±	100.2 ±	100.4 ±	99.8 ±
± SD*	1.5	0.7	0.3	0.6	0.4	0.5	1.1	0.8	0.7	1.5	0.9	0.4
C.V**	1.5	0.7	0.3	0.6	0.4	0.5	1.1	0.8	0.7	1.5	0.9	0.4

* Average of six replicates.

** Coefficient of variation.

• Results are calculated according to standard curves.

Table [2]: Accuracy of the proposed spectrophotometric for analysis of the studied PPIs at three concentration levels.

3.3.2- Accuracy: Accuracy was checked by applying the proposed spectrophotometric method for the assay of the studied PPIs at three concentration levels. Recoveries were in the range from 99.5- 100.5 % ± 0.4-1.5, Table (3).

3.3.3- Precision: Results of interday and intraday precision were studied for the proposed method. Results showed that the relative standard deviations were less than 1.5 % in all cases, indicating good repeatability of the proposed spectrophotometric method, Table (4).

3.3.4- Robustness [43]**:** Slight variation in several assay parameters such as concentration and volume of p-nitroaniline, sodium nitrite as well as sodium hydroxide, volume of diazonium salt and reaction time was found not to significantly affect performance of the proposed method. This confirms that the proposed method is robust**,** Table (5).

Authentic drug	Concentration (µg mL^{-1})	Intraday precision		Interday precision	
		% Recovery ± SD*	C.V**	% Recovery ± SD*	C.V**
OMZ	10	100.6 ± 1.4	1.4	98.5 ± 0.5	0.5
	20	100.2 ± 0.6	0.6	99.5 ± 0.7	0.7
	30	99.9 ± 0.3	0.3	100.0 ± 0.4	0.4
LAN	10	100.6 ± 0.5	0.5	100.4 ± 0.8	0.8
	20	99.5 ± 0.3	0.3	99.4 ± 0.6	0.6
	30	99.8 ± 0.7	0.7	99.8 ± 0.3	0.3
PAN	10	100.0 ± 1.0	1.0	100.9± 1.2	1.2
	20	99.3 ± 0.8	0.8	99.7 ± 0.7	0.7
	30	99.6± 0.4	0.4	99.1 ± 0.8	0.8
RAB	10	101.4 ± 1.3	1.3	99.1 ± 0.7	0.7
	20	100.9 ± 0.8	0.8	99.9 ± 0.8	0.8
	30	100.1 ± 0.2	0.2	99.6 ± 0.6	0.6

* Average of six replicates.

** Coefficient of variation.

Table [3]: Interday and intraday precision of the proposed spectrophotometric method for analysis of the studied PPIs at three concentration levels.

Variation	% Recovery ± SD*			
	OMZ**	LAN**	PAN**	RAB**
No variation•	101.2 ±1.21	99.2 ±1.11	99.6 ±0.32	102.3 ±1.34
p-nitroaniline conc.				
0.30 g % w/v	98.8± 0.81	99.7± 1.21	101.2± 1.20	98.0± 0.61
0.34 g % w/v	99.7± 0.87	97.8± 0.98	99.1± 0.67	99.1± 0.76
$NaNO_2$ conc.				
0.38 g % w/v	101.3± 1.30	98.4± 1.31	98.3± 1.10	102.3± 1.13
0.42 g % w/v	102.3± 0.89	101.2± 0.98	99.1± 1.42	100.2± 0.87
Hydrochloric acid				
23 mL	97.3± 1.23	97.5± 1.21	98.5± 1.23	98.7± 0.92
27 mL	98.1± 1.12	97.3± 0.88	99.1± 0.99	103.1± 1.50
Diazonium salt volume				
1.4 mL	98.3± 1.10	102.1± 1.21	98.8± 0.70	98.3± 0.43
1.6 mL	98.5± 1.23	99.2± 0.88	98.2± 1.11	100.0± 0.92
NaOH conc.				
1.4 N	99.3± 1.41	103.1± 0.75	97.8± 0.89	101.2± 1.10
1.6 N	102.3± 0.98	99.2± 0.33	98.1± 1.02	99.2± 1.34
NaOH volume				
0.75 mL	102.3± 1.41	99.3± 1.35	98.5± 0.98	98.3± 1.20
0.85 mL	100.2± 1.30	98.2± 1.13	100.2± 1.21	99.2± 1.09
Reaction time				
6.5 min.	99.9± 1.20	99.9± 0.67	98.8± 0.91	100.9± 0.50
7.5 min.	98.3± 0.65	99.3± 1.34	99.8± 1.23	98.7± 1.25
Stability time				
85 min.	100.1± 0.63	100.5± 1.21	101.3± 1.31	102.0± 1.34
95 min.	99.2± 0.92	99.2± 1.45	98.3± 0.91	99.2± 0.99

* Average of five replicates. ** Drug concentration = 20 µg mL^{-1}

• No variation in procedure parameters.

Table [4]: Robustness of the proposed spectrophotometric method.

3.3.5- Application to pharmaceutical dosage forms: The proposed spectrophotometric method was applied for the assay of pharmaceutical dosage forms Table (6). Results obtained agree well with those of the reported method [44] based on t- and F- tests at 95% confidence level.

Recovery studies were also performed by using standard addition method [45], Table (7).

3.4- Investigation of the reaction mechanism

3.4.1-Stoichiometry of the reaction: Job's method of continuous variation [46] revealed that the maximum absorption occurred when the molar ratio of the measured product is 1:1 (drug: diazotized p-nitroaniline), Fig. (3)

Authentic drug	Pharmaceutical formulation	% Recovery± SD*		t-value[b]	F-value[b]
		Proposed	Reported[a]		
OMZ	Omepak® capsules	100.8±0.5	99.2±0.5	0.453	1.004
	Losec® tablets	100.5±0.3	98.8±0.5	0.936	0.370
	Risek® vials	99.6±0.6	100.0±0.6	0.731	1.822
LAN	Zollipak® capsules	99.1±0.8	99.1±0.5	0.267	4.568
PAN	Pantoloc® tablets	101.5±0.5	100.7±0.6	0.684	0.589
	Pantazol® vials	99.1±0.3	99.8±0.6	0.449	0.214
RAB	Rabicid® tablets	100.8±0.3	100.1±0.4	0.319	1.489

* Average of six replicates.

[a] Reference [44].

[b] Theoretical value for t and F at 95% confidence limit, t= 2.228 and F=5.053.

Table [5]: Application of the proposed spectrophotometric method to pharmaceutical formulations.

Authentic drug	Pharmaceutical formulation	Amount added (mg)	Amount found (mg)	% Recovery± SD*
OMZ	Omepak® capsules	5	4.94	98.8±0.69
		10	9.68	96.8±0.97
		15	15.07	100.5±0.95
		20	20.01	100.1±0.32
	Losec® tablets	5	5.18	103.6±0.38
		10	9.57	95.7±0.70
		15	14.66	97.8±0.70
		20	19.88	99.4±0.10
	Risek® vials	5	5.13	102.6±0.72
		10	9.51	95.1±1.11
		15	14.55	97.0±0.85
		20	19.90	99.5±0.55
LAN	Zollipak® capsules	5	4.77	95.4±0.24
		10	9.85	98.5±0.28
		15	15.08	100.5±0.28
		20	20.15	100.8±0.34
PAN	Pantoloc® tablets	5	4.92	98.2±0.69
		10	9.69	96.9±0.69
		15	14.48	96.5±0.91
		20	19.50	97.5±0.51
	Pantazol® vials	5	4.89	97.9±0.54
		10	10.00	100.0±0.76
		15	14.70	98.0±0.45
		20	19.65	98.3±0.78
RAB	Rabicid® tablets	5	4.88	97.5±0.46
		10	9.88	98.8±0.69
		15	14.79	98.6±0.60
		20	19.70	98.5±0.67

* Average of five replicates.

Table [6]: Analysis of pharmaceutical formulations by the proposed method using standard addition method.

Chemical shift (ppm), δ-values	Number of protons (multiplicity)	Assignment
3.76	3(s)	a-OCH_3
3.93	3(s)	b-OCH_3
4.50	1(m)	c- CH
6.75	1(m)	d-CHO
7.05	1(d)	e-CH
7.10	1(d)	f-CH
7.00	1(s)	g-CH
7.45	1(d)	h-CH
8.05	1(d)	i-CH
7.25	1(d)	k-CH
7.35	1(d)	m-CH
8.25	1(d)	l-CH
8.40	1(d)	n-CH
6.8	1(s)	o-NH

Table [7]: Chemical shifts and multiplicity of protons of the isolated reaction product.

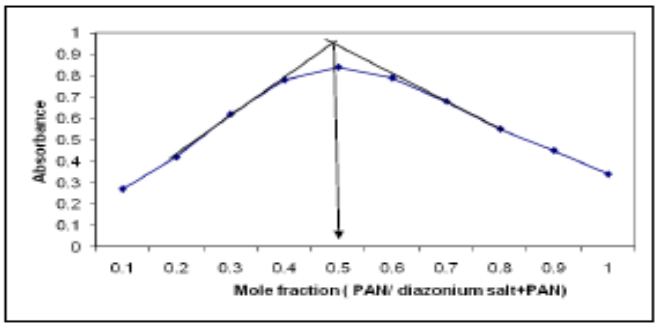


Figure [3]: Job's plot of continuous variation of PANwith diazotized p-nitroaniline.

3.4.2- UV/VIS spectra: The UV-VIS absorption spectrum of the methanolic solution of PAN (λ_{max}= 295 nm) was found to be different from that of the isolated product (λ_{max}= 610 nm), Fig. (4).

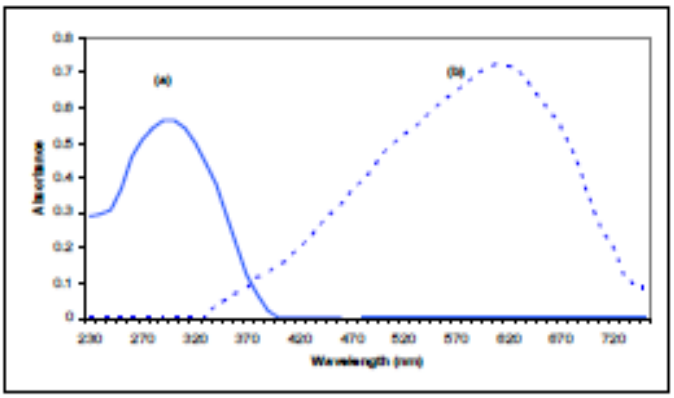


Figure [4]:- UV-VIS spectra of PAN (a) and the isolated reaction product(b).

3.4.3- IR- spectra: The IR spectrum of the isolated reaction product was compared with that of PAN [47]. It was evident from the obtained spectra appearance of -N=N- in the product as indicated by the sharp signal at 1606 cm^{-1} which is not originally found in the spectrum of PAN. Besides, disappearance of the signal at 829 cm^{-1} is indicative of the CH_2 out-of-plane bending in PAN Fig. (5).

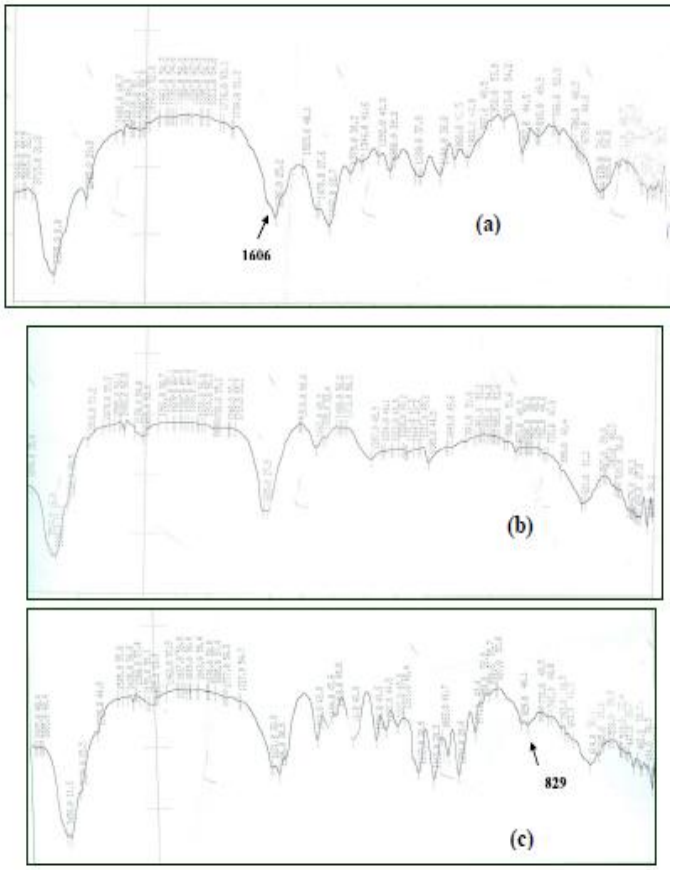


Figure [5]: IR- spectra of reaction product (a), p-nitroaniline (b) and PAN (c).

3.4.4-NMR- spectrum: NMR-spectrum of the isolated product in deuterated dimethylsulfoxide 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 protons in the product. Signals observed at δ, 2.50 is characteristic band for DMSO and that observed at δ, 3.50 is also characteristic for the water present in the solvent, Fig.(6).

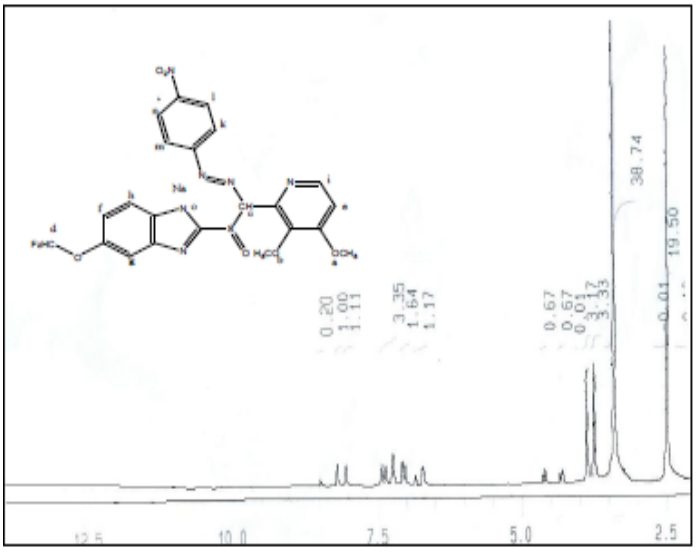
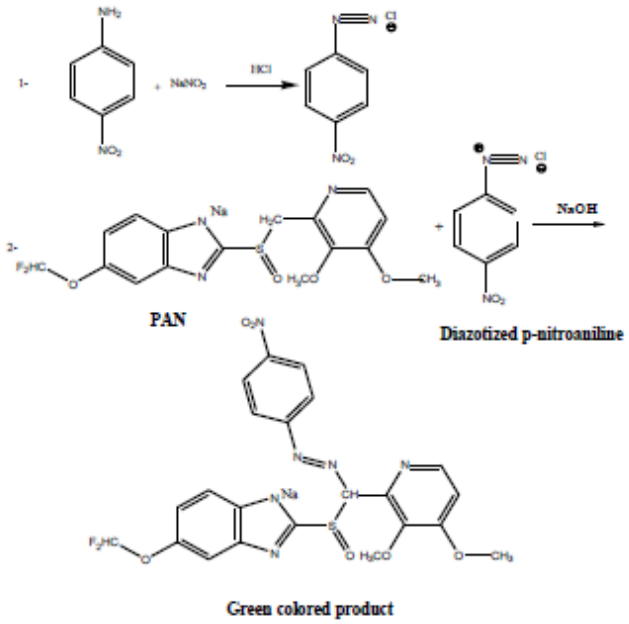


Figure [6]: NMR- spectrum of the isolated reaction product.

3.4.5- Reaction mechanism: From the above spectral data, we can, with a high degree of certainty, suggest the following mechanism of the reaction (scheme 2):



Scheme [2]: The suggested reaction mechanism betwen PAN and diazotized p-nitroaniline.

4- Conclusion:

A rapid, simple and validated spectrophotometric method was developed for the assay of PPIs (LAN, PAN and RAB). Mechanism of reaction; based on coupling with diazotized p-nitroaniline; was suggested using IR and NMR spectra of the isolated reaction product. Statistical analysis proves that the developed method was accurate, and repeatable for the analysis of the studied drugs in their pure forms and in pharmaceutical formulations and can be used for routine quality control analysis of the investigated drugs.

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Conflict of Interest: None Declared

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