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A simple and sensitive colorimetric method for the determination of Propranolol hydrochloride in pure and pharmaceutical preparation via oxidative coupling organic reaction

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Abstract

Precise, rapid and simple spectrophotometric method for the estimation of Propranolol hydrochloride (PRO) drugs has been developed. This method is based on an oxidative coupling reaction between above drugswith 1,4-diamiobenzene reagent solution in a basic medium (pH 11.17) in the presence of N-Bromosuccinimideto production anorange colour, stable,soluble in water and gave absorption at 463 nm.With correlation coefficient 0.998 Beer's law isobeyed in the linear range (2.5-0.75)µg/ml of (PRO),thedetection limit, Sandell's sensitivity andthe molar absorptivity were 4.229 µg/ml, 0. 09µg.cm^{-2} and3.283x10 ^{3}liter. mol^{-1}.cm^{-1} respectively while RSD value andrecovery were2.02%,100.007.% The proposed method was plate good success to the estimation of (PRO)drug in tablets.

Keywords: spectrophotometric, Propranolol hydrochloride (PRO) drugs,1,4-diamiobenzenereagent

INTRODUCTION:

Propranolol hydrochloride (PRO)is a non-selective beta blocker. It is chemically structure (RS)-2-(4-(2-methylpropyl) phenyl) 2-Propanol, 1-[(1-methylethyl) amino]-3-(1-naphthalenyloxy), hydrochloride (Figure 1) [1,2].{$C_{16}H_{21}NO_2$, HCl}[3,4]. is an antihypertensive agent (PRO)is used in the manipulation or avoiding of many troubles including hypertensive emergencies, hyperthyroidism,acute myocardial infarction, angina pectoris, hypertension,anxiety, pheochromocytoma,arrhythmias, menopause, and, migraine[5-8]. This beta-blocker may work by stabilizing arteries or avoiding the central alternator of migraine in the brainstem from firing.(PRO)drugs is official in British Pharmacopoeia and United States Pharmacopoeia [9-11]. Due to its therapeutically and pharmacological properties, many analytical methods have been developed for determinationof these drugs includes spectrophotometry[12-14].,colorimetric [15], fluorimetry[16], voltammetry[17], gas chromatography-mas-spectrometry (GC-MS) [18,19], high-performance liquidchromatography (HPLC) [20-22], liquid chromatography-massspectrometry (LC-MS) [23,24], chemiluminescence [25,26],capillary electrophoresis [27,28] and Titrimetric method[29,30].Visible spectrophotometry methods, because of their inherent simplicityand rapidity of the procedure and low cost-effectiveness,sensitivity,selectivity and fair accuracy and precision of the techniques findfavour in most laboratories of limited means and consequently thesemethods continue to flourish.The proposal method is adopted on thereaction of the Propranolol hydrochloride drug with1,4-diamiobenzenein the presence ofN-Bromosuccinimidetoform an orange water soluble colour productin alkaline mediumwhich gave an absorption at λ_{max}= 463 nm.

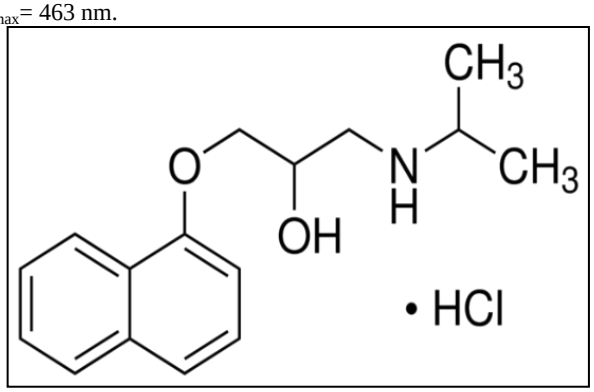


Figure 1: Structure of Propranolol hydrochloride

EXPERIMENTAL

Apparatus

- UV-visible, shimadzu 1700
- spectrophotometer, with1.0 cm quartzcells was used for absorptionmeasurements,
- WTW 720 pH meter.
- Electronic balance,Kernacj/ Germany,ACS..

Reagents

All chemicals and analytical reagents are high purity and supplied by companies SIGMA,CDH,Fiuke and S.D.I.

Preparation of solutions

A-Standard PRO solution, (1000µg/ml):

The stock solutionof(PRO)was prepared by dissolving 0.1 gmof(PRO)in 10ml ethanol and diluting bydistilled water to the mark100 ml volumetric flask. Workingsolutions were prepared by diluting thesolution in distilled water.

B- 1,4-diamiobenzene reagent solution (1x10^{-2} M):

This solution was prepared by dissolving 0.11 g of 1,4-diamiobenzenein distilled water and diluting to mark in the volumetric flask to 100 ml distilled water.

C- N-Bromosuccinimide (1x10^{-2} M):

By dissolving 0.177 gm of N-Bromosuccinimidein 5 ml acetone and diluting to the mark100 ml volumetric flaskThis solution was prepared

D - Sodium hydroxide solution (1.0 M):

The NaOH solution was prepared by weighing 4.000 g of pure sodium hydroxide and dissolving in100 ml of distilled water and then calibration with standard hydraulic acid

E-The PRO tablets formulationSolution (500 µg/ml):

Pharmaceutical formulation of PRO (producedbyAccordHealthcare Ltd), the tablet contains 40 mg of PRO and the solution was prepared by following method: Fourteen tablets was weighed (0.2724g) and grinding well, then a weight of 0.34 g of the powder and dissolved in 15 ml of ethanol, The solution filtered by paper filtration, in100 ml volumetric flask the volume was completed with distilled water.

Preliminary Investigations:

A 1ml of N-Bromosuccinimide(1x10^{-2} M) was added to 2ml of standard PRO(200µg/ml) solution and then add 1 ml of 1,4-diamiobenzenereagent (1x10^{-2} M) in basic medium (1ml of 1M, Sodium hydroxide),in a 10 ml volumetric flask the solutionwas diluted with distilled water, anorange Color soluble product. the Colored dyeshows maximum absorption at 463 nm against its corresponding blank reagent whilethe blank reagent shows no absorbance at same wavelength.

Optimization of the experimental conditions:

The influence of different variables on intensity of the colour of1ml of N-Bromosuccinimide($1x10^{-2}$ M) , 2ml of standard PRO(200µg/ml) solution in the presence of 1ml of1,4-diamiobenzenereagent ($1x10^{-2}$M) in basic medium (1ml of 1M, Sodium hydroxide), was studied to establish the optimum conditions.

Selection of the coupling reagent

Different types of coupling reagents are investigated to select the best reagent that gives the highest color intensity, the results are shown in Table(1).

Table (1) Selection of the coupling reagent.

Reagent 1×10^{-3} M	Variable	Absorbance	λ_{max} (nm)	$\Delta\lambda_{max}$ (nm)	.mol^{-1}.cm^{-1}Ɛ.L
1,4-diaminobenzene	**B**	**1.32**	**272**	**191**	**8.615×10^3**
	S	0.112	463		
Catechol	B	1.27	260	200	7.153×10^3
	S	0.093	460		
4-Aminoantipyrine	B	0.72	359	86	19.23×10^3
	S	0.25	445		
Ansidine	B	.....	.....	.....	.....
	S	.....	.....		

$\Delta\lambda_{max} = \lambda_{max}$ (S) – λ_{max} (B)

S = Sample Vs. Blank

B = Blank Vs. water

The results illustrated in Table (1) indicate 1,4- diaminobenzene reagent gives the highest color intensity and a good color contrast Δλ in comparison with other reagents. so this reagent is chosen in later experiments.

Selection of the oxidizing agent

The effect of the oxidizing agent was studied by adding 1 ml of various types of oxidizing agents (1×10^{-2}M) to 2 ml of PRO(200µg/ml)solution, 1ml of1,4-diamiobenzenereagent ($1x10^{-2}$ M).and 1ml of sodium hydroxide solution(1M). The results are shown in Table (2).

Table (2) Selection of the oxidizing agent.

Oxidizing agent 5×10^{-3}M	Absorbance	λ_{max} (nm)	.mol^{-1}.cm^{-1}Ɛ.L
N-Bromosuccinimide	**0.112**	**463**	**8.615×10^3**
Iron chloride	0.102	512	7.846×10^3
Sodiumnitroprusside	.....	.....	……
Sodium metaperiodate	.....	.....	.....
Potassium iodated	.....	.....	……..

The N- Bromosuccinimide solution shows a higher absorption for orange product at a λ_{max} = 463 nmwhen compared with other oxidizing agents, so N- Bromosuccinimide is select in subsequent experiments.

Effect of pH

To study the effect of pH it was added 0.5-4.0 ml of 1.0 M various bases. Sodium carbonate was the best base, pH is found to be 11.17, so the pH of 11.17 was adopted in subsequent experiments, the results are shown in Table (3). It is worth noting that no color was obtained on the addition of any amount of acid indicating that no reaction is occurred.

Effect of the amount of coupling reagent

The effect of the amount of 1,4-diamiobenzene reagent was examined by following method, In volumetric flask contains 2.0 ml of PRO(200 µg/ml)and 1ml of N-Bromosuccinimide($1x10^{-2}$ M) addedvarious volumes (2-0.25 ml) of 1,4-diamiobenzenereagent (1×10^{-2} M), andthen added 1.0 ml of 1.0 M sodium carbonate andwith distilled water the volume is completed to 10ml,The Table (4)is show the results.

Table (3) Effect of pH

Volume(ml) 1M)(	NaoH		$Na2CO_3$		NH_4OH		NaHCO3	
	Abs	pH	Abs	pH	Abs	PH	Abs	pH
0.5	0.261	11.81	0.337	10.89	0.130	9.98	0.06	9.18
1	0.251	12.01	0.344	11.08	0.138	10.13	0.065	9.24
1.5	0.245	12.11	**0.358**	**11.17**	0.161	10.28	0.083	9.33
2	0.241	12.23	0.340	11.23	0.167	10.41	0.09	9.44
2.5	0.231	12.30	0.331	11.31	0.187	10.56	0.105	9.54
3	0.225	12.39	0.320	11.44	0.209	10.63	0.110	9.61
3.5	0.221	12.46	0.280	11.56	0.221	10.73	0.118	9.7
4	0.215	12.60	0.270	11.70	0.245	10.81	0.124	9.85

Table (4) Effect of the amount of coupling reagent.

ml of 1,4 -diaminobenzene (1×10^{-2}M)	Absorbance
0.25	0.125
0.5	0.198
0.75	0.255
1	**0.288**
1.25	0.244
1.5	0.228
1.75	0.218
2	0.205

From the Table (4) the best volume of 1.0ml of 1,4-diamiobenzene (1×10^{-2}M) was the best amount of coupling reagenttherefor it is chosen in later experiments

Effect of the amount of oxidizing agent

To investigate the best amount of oxidizing agent N-Bromosuccinimide($1x10^{-2}$ M) this study was conducted by the following method,added different volumes (0.5-2.5 ml) of N-Bromosuccinimideto 2.0 ml of PRO(200µg/ml) and 1,4-diamiobenzene in volumetric flasks (10 ml)then addition of 1.0 ml of 1.0M sodium carbonate andwith distilled water the volume was completed to 10ml, Table (5).

Table (5) Effect of the volumeof oxidizingagent.

ml of N – Bromosccucinimide (1×10^{-2} M)	Absorbance
0.5	0.147
0.75	0.221
1	0.235
1.25	0.250
1.5	**0.266**
1.75	0.251
2	0.243
2.25	0.230
2.5	0.219

Table (5) shows that the volume of 1.5ml of N – Bromosccucinimide solution(1×10^{-2}M) is the best amount, so it was used in later experiments.

Effect of oxidation time

1.5 ml of N-Bromosuccinimide($1x10^{-2}$ M) , 2ml of standard PRO(200µg/ml) solution added to a series of volumetric flasks, left this solutions for different periods of

time, then 1ml of1,4-diamiobenzenereagent ($1x10^{-2}$M) and1.5 ml of 1 M Na_2CO_3 solution were added. with distilled water the volume was completed to 10 ml, measured the absorption of solutions at λ_{max} = 463 nm versus blank, Table (7).

Table (7) Effect of oxidation time.

Time(min)	Absorbance
5	0.241
10	0.29
15	0.34
20	0.354
25	0.365
30	**0.377**
35	0.369
40	0.361
50	0.341
55	0.334

From Table (7), 30 min is sufficient time for the oxidation, so it is utilized in the later experiments**.**

Effect of temperature

The influence of temperature(5-50°C) ondetermined color intensity of the formed yield wasexamined by adding 2 ml of PRO(200µg/ml)and 1.5 ml of N-Bromosuccinimide($1x10^{-2}$ M)then1,4-diamiobenzenereagent ($1x10^{-2}$M), 1.5 ml of 1 M sodium carbonate solution were added, thenwith distilled water the volume is diluted to the mark in10 mlof series volumetric flasks, at λ_{max} = 463 nm the absorption was measured versus blank, Table (8).

Table (8)Effect of temperature.

Temp C^O	Absorbance
5	0.291
10	0.302
15	0.363
20	0.377
25	**0.376**
30	0.373
35	0.343
40	0.284
45	0.260
50	0.252

Table (8) show that the (15-30)°C is the best temperature , so 25°C isutilized in the later experiments.

Effect of stability time on the colored product

To study stability time of the coloredcompound taking 1.5 ml of N-Bromosuccinimide($1x10^{-2}$ M), 2ml of standardPRO (200µg/ml) solution, then 1ml of1,4-diamiobenzenereagent ($1x10^{-2}$M) and 1.5 ml of 1 M Na_2CO_3 solution were added.with distilled water the volume is diluted to the mark in10 ml of series volumetric flasks. After dilution the absorption remain unchanged for 60 minutes. Table (9).

Table (9) Effect ofstability time on the colored product.

Time(min)	Absorbance		
	20µg/ml	40µg/ml	50µg/ml
5	0.121	0.352	0.483
10	0.114	0.339	0.462
15	0.114	0.331	0.453
20	0.110	0.318	0.438
25	0.109	0.309	0.421
30	0.107	0.302	0.415
35	0.106	0.299	0.413
40	0.104	0.291	0.406
45	0.098	0.283	0.404
50	0.096	0.274	0.401
55	0.094	0.272	0.398
60	**0.093**	**0.271**	**0.395**

Effect type of the solvents

The influence of the solvents on the product (coloredcompound)was investigated,instead of water the dilution was perform byadding different organic solvents. Table (10).

Table (10) Effect of the solvents.

Solvent	λ_{max}	Absorbance
Water	**463**	**0.353**
Ethanol	482	0.238
Methanol	473	0.209
Acetone	480	0.215

From the Table (10) the water gavebest absorption at the 463 nm therefor it has been used as the best solventin the later experiments.

Effect of Order of additions:

Theinfluenceofreagents addition orders on the absorption of the orange productwereinvestigated. From Table(11) the addition in sequence (3) accomplishes a best absorption of orange product, therefore it is utilized in later experiments.

Table (11) Effect of Order of additions

NO	Order of additions							Absorbance
1	D	+	O	+	R	+	B	0.371
2	D	+	R	+	O	+	B	0.046
3	**R**	**+**	**O**	**+**	**D**	**+**	**B**	**0.381**
4	O	+	D	+	R	+	B	0.321
5	R	+	O	+	B	+	D	.....

D = Propranolol HCl

R = 1 , 4 – Diaminobenzene

O = N – Bromosuccinide B = Sodium Carbonate Na_2CO_3

The final absorption spectrum

The final spectrum of the orange product by oxidative coupling reaction of PROwith1,4-diamiobenzenereagent ($1x10^{-2}$M) in the presence of N-Bromosuccinimide **(**$1x10^{-2}$ M) in temperature 25°C andbasic medium verses reagent blank show a maximum absorption at 463 nm while the blank reagent gave zero absorbance at λ_{max}. This spectrumis shown on Fig. (2).

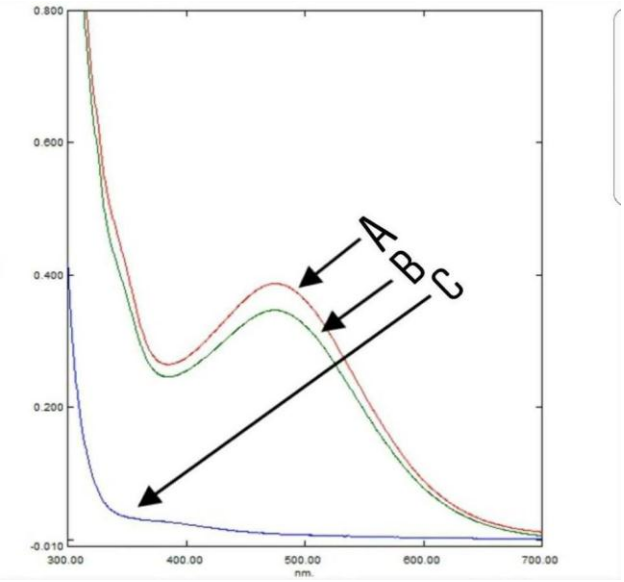


Fig. (2) Final absorption spectrum of the determination PRO.

A:PRO solution versus blank reagent.

B: PRO solution versus distilled water.

C: Blank reagent versus distilled water.

Procedure for construction of calibrationcurve

Toconstruct linear calibration curve of the estimation of PROin the range2.5-0.75ml,1 ml of PRO(200 µg/ml) were transferred,1.5ml of N-Bromosuccinimide(1×10^{-2}M) and 1mlof1,4-diamiobenzenereagent (1×10^{-2}M), 1.5 ml of 1 M sodium carbonate (pH 11.7)were added at 25°C . Solutions were left for 10 min to complete the reaction, then the volumes were diluted to the mark with distilled water. The absorption was measured at 463 nm verses the blank. Fig. (3)

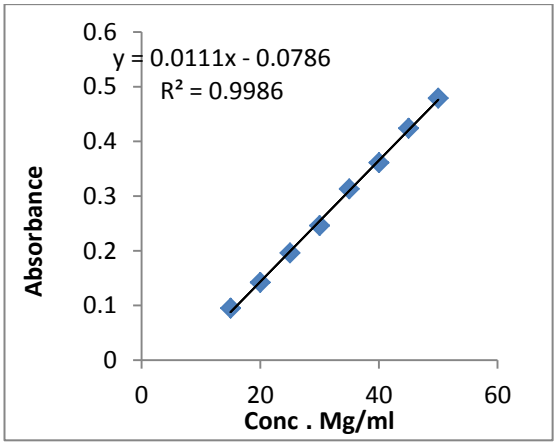


Fig. (3) Calibration curve for determination PRO by oxidativecoupling with 1,4-diamiobenzenereagent.

Accuracy and precision

For three different concentrations of thePRODrug(20,30,40)µg/mltheabsorption wasmeasured(six times) at 463 nm and Accuracy and precision were calculated,the average recoveryand the relative standard deviation were (100.002 %) (<0.19%). The results Table (11) indicate that the method is of high accuracy and precision.

Table (11) Results of accuracy and precision.

Conc. 0f PRO [Mg/ml]	RE %	Recovery %	Average recovery %	RSD %
20	0.0002	100.0002	100.001	1.98
30	0.002	100.002		1.403
40	0	100		0.97

Detection limit

Detection limit was calculatedby takingthe lower concentration 15 µg/ml at optimal conditions (six times) at 463 nm and measuring the absorption. Table ((12)

Table (12) Detection limit.

Concentration [Mg/ml]	x —	S	D.L Mg/ml
15	0.1098	0.01032	4.229

The nature of the colored product

Slop ratio method and mole ratio method were applied to detect the nature of the orangedye product (stoichiometry ofPRO drug with the reagent). In these methods, both of the standard PRO solution and 1,4-diamiobenzenereagent solution were equal concentration (1×10^{-2}M). In slop ratiomethod: by mixingdifferent volumes of the drug solutions (0.1-0.9) ml and different volumes(0.1-0.9) ml of1,4-diamiobenzene reagent solution in a series of volumetric flasks (10ml), A 1.5 mlofN-Bromosuccinimide(1×10^{-2} M) and 1.5 ml of 1 M sodium carbonate solution were added andto the mark with distilled water the volumes were completed. The absorptions were measured at 463 nm versus the blank reagent. Fig. (4) show the ratio was 1:2

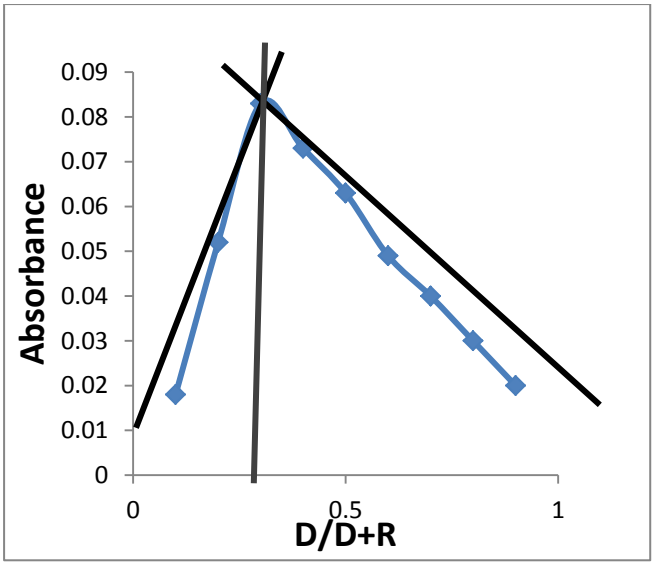


Fig. (4) Job's method of formed product by oxidativecoupling of PRO with 1,4-diamiobenzenereagent.

In molar ratio method,in a series of volumetric flasks (10 ml) 1 ml of the standard drugand different volumes 0.2-2.0 ml of 1,4-diamiobenzenereagent solution were transferred, then adding1.5 mlofN-Bromosuccinimide(1×10^{-2} M) and 1.5 ml of 1 M sodium carbonate solution.To the mark with distilled waterthe volumes were completed,at 463 nm the absorption was measured versus the blank reagent. FromFig. (5) themolar ratio was 1:2. The results were agreement with the Job's method results.

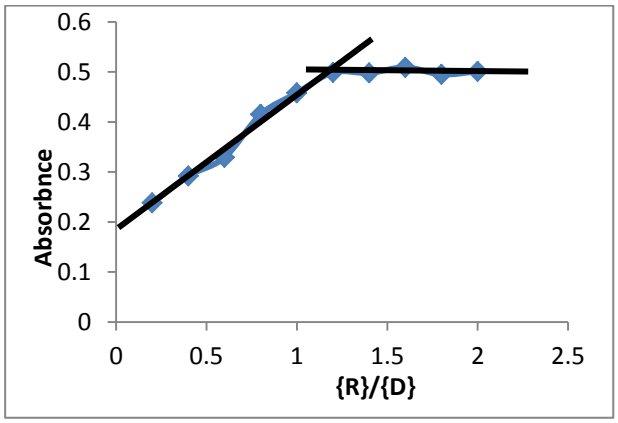
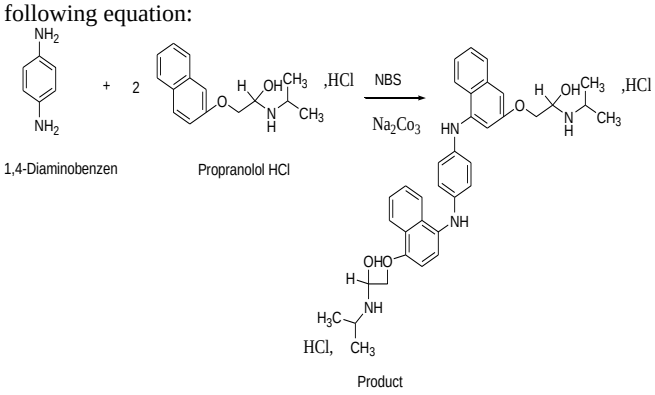


Fig. (5) Molar ratio method for the product formed by oxidativecoupling of PRO with 1,4-diamiobenzenereagent

The formation of the color product may probably occursas following equation:



Applications

This method was applied for the determination of PRO in its pharmaceutical formulation (production of Accord Healthcare Ltd).

Direct method

In 10 ml volumetric flaskstransferred different volumes (1.0,1.5, 1.75ml) of a pharmaceutical formulation solution(200 µg/ml), the resulting concentrations (20,30,35) µg/ml treated as in construction of calibration curve thenat 463 nm the absorbance was measured for six times.calculated Recovery and RSD Table (13) show the results.

Table (13): Direct method

Conc. Of PROµg/ml	RE %	Recovery	Average recovery%	RSD %
20	0.0004	100.0004	100.0002	9.37
30	0	100		1.7
35	0.0004	100.0004		1.20

Results from the above tablethe value of the recovery of 100.0007% in the product (production of Accord Healthcare Ltd) indicate the success ofthe proposed method to determinate PRO in itspharmaceutical preparation.

Standard additions method:

To prove that the proposed method is free from interferences.Thestandard additions methodwas applied for estimating of PRO in its pharmaceuticalpreparation,in seven volumetric flasks (10 ml) for each volumetransferreddifferent volumes (1-1.2)ml of a pharmaceutical formulation solutions (200 µg/ml), then increasing volumes (2.2 ,1.8 ,1.4, 0.6)mlof 200 µg/ml of PRO standard solution were added with leaving the seventh flask without addition. The solutions were treated by usingoptimized conditions.at 463 nm.The absorptions were measured (Fig.6) the measured concentration was calculated from the equation of the straight line and the results of Recovery areshown in the Table (14).

Table (14): Standard additions method

Type of Drug	PROpresent µg/ml	PROmeasured µg/ml	Recovery,(%)
Tablets PRO (40 mg) accord	12	8.57	100.007
	28	23.81	100

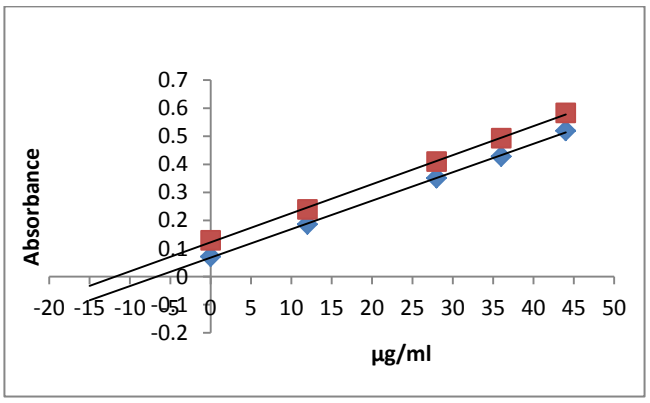


Fig (6): Standard additions method

The results in Table (14) indicate that theStandard additions methodis in agreement with the direct method within the acceptable range of error, therefore that the proposed method isaccepted and free from interferences

CONCLUSIONS

The developed method isaccurate, preciseand selectivefor the estimation of PRO. It's based on oxidative coupling reaction between PRO and 1,4-diamiobenzenereagent in presence of N-Bromosuccinimidein basic mediumto form orange colored productwhich isstable, water soluble shows a maximum absorption at 463 nm. The proposed method can be carried out with no need for furthersteps such as solvent extraction step, pH or Temperature control and it can be applied successfully for in estimation of (PRO) drug in tabletsandpharmaceutical formulation.

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