



## Development of Two Simple Spectrophotometric Methods to Assay Phenylephrine-HCl as Pure Form and in its Drug Forms

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**Abstract:** This work includes the development of two spectrophotometric methods which are sensitive, accurate, stable, and has good recovery for the determination of phenylephrine-HCl as free form and in the pharmaceutical preparations. In the method (A), phenylephrine is oxidized by potassium permanganate in a basic solution of sodium hydroxide and the bluish-green color of the resulting manganate ( $\text{MnO}_4^{2-}$ ) is measured at wavelength 610 nm, which is linearly proportional to the amount of phenylephrine-HCl. Method (B), is involved the oxidation of phenylephrine-HCl by using an excess amount of N-bromosuccinimide in an acidic medium of hydrochloric acid solution, the remaining (unreacted) amount of N-bromosuccinimide is used to bleach indigo carmine dye and the absorbance of the blue color of the remaining dye is measured at the wavelength of 610 nm. which is directly proportional to the concentration of phenylephrine-HCl. The molar absorptivity coefficients of methods (A) and (B) are estimated and equal to  $1.5722 \times 10^4$  and  $5.5191 \times 10^4$  L/mol.cm, respectively. Beer's law of the both methods are linear in the concentration ranges 0.2–8.0  $\mu\text{g/mL}$  (method A) and 0.2–3.5  $\mu\text{g/mL}$  (method B). The relative standard deviation values of methods (A) and (B) are also found to be better than 0.0286 and 0.0114, respectively. The two proposed methods are applied to estimate phenylephrine-HCl in injection and drop.

**Keywords:** Indigo carmine,  $\text{KMnO}_4$ , NBS, Oxidation, Phenylephrine-HCl, Spectrophotometry.

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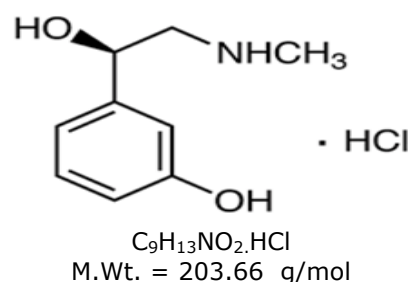
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### 1. INTRODUCTION

Phenylephrine-HCl (PPH) represents a type of sympathomimetics drugs that directly affects and stimulates alpha-1 adrenergic receptors found in blood vessels and smooth muscles (1). PPH is usually indicated to raise blood pressure in unstable patients with hypotension, especially causes from septic shock, and its nasal drops are used to treat symptoms such as itching of the nose and throat, sneezing and runny nose (2).

Chemically, PPH is (R)-1-(3-hydroxyPHEPHnyl)-2-methyl amino ethanol hydrochloride with chemical formula  $\text{C}_9\text{H}_{13}\text{NO}_2 \cdot \text{HCl}$ . It is a white crystalline powder having good solubility in alcohol and water with freely degrees. Scheme (1) explain the chemical structure of PPH (3).



**Scheme 1:** The chemical structure of PPH.

PPH has been determined by using a wide variety of analytical techniques, these methods includes: RP-high performance liquid chromatography (4,5,6), high-performance thin layer chromatography (7,8) and RP-HPLC-PDA method (9), voltammetry (10,11), conductimetric titration (12), flow injection analysis (13,14), ultraviolet spectrophotometry (15,16), renewable electrode of carbon nanotubes ceramic electrode (17) and derivative spectrophotometric method (18) have also been reported for the determination of PPH.

The technique of UV and visible spectrophotometry is mostly used for the determination of PPH in aqueous medium because it offers advantages of simple and low cost instrument that are available at all laboratories. Most researches are described utilizing spectrophotometric methods for estimating PPH in the bulk and in its pharmaceutical formulations using different reactions and various reagents. Some of these methods involve diazotization and coupling reaction (19-24). Other methods base on the oxidative coupling reaction of PPH with 4-aminoantipyrine and potassium ferricyanide to yield dirty ping water soluble product (25), N,N-dimethyl-p-phenylenediamine in the presence of sodium persulfate in basic medium to give panic solution soluble product with maximum absorbance at 502 nm (26), and N,N-dimethyl-p-phenylenediamine dihydrochloride with ferric chloride in basic media to form green-blue soluble dye product (27,28). Other spectrophotometric methods based on the oxidation of PPH either with an excess amount of chloramine-T and the residual NBS is determined by bleaching the colour of indigo carmine dye (29) or by adding an excess amount of N-bromosuccinimide and then the residual NBS is estimated by bleaching the color of methyl orange dye (30). However, many of these methods suffer from various limitations, for instance, low sensitivity, low stability of the resulting product, and long operating time. Others required solvent extraction and expensive devices which may not be present in the laboratory. The aim of this current work describes two development spectrophotometric methods (direct and indirect) for assaying PPH in the bulk and in its pharmaceutical forms (injection and drop).

## 2. EXPERIMENTAL

### 2.1. Instrumentation

A Jasco V-630 digital double beam UV-Vis. spectrophotometer equipped with 1.0-cm matched fused quartz cells and Bp3001 professional bench top pH meter devices are used for all absorption spectra recording and pH measurements, respectively.

### 2.2. Chemical Reagents and Standard Solutions

All analytical reagents and chemicals used are of a high degree of purity, obtained from approved international and local origins. The standard material of PPH is procured from the state company for drug industries (SDI), Samarra- Iraq.

*Standard Solution of PPH (100 µg/mL) ( $4.910 \times 10^{-4}$  M):* It is prepared by weighing 0.0100 g of PPH and dissolving it in an appropriate volume of distilled water (Dw), and the solution is then transported to a volumetric flask of 100 mL and made it to the mark with Dw.

*Working Solution of PPH (20 µg/mL) ( $1.196 \times 10^{-4}$  M):* An appropriate volume of the standard solution of PPH is diluted with Dw to obtain the working solution.

*Stock Solution of Potassium Permanganate (0.06 N):* 0.3793 g of pure potassium permanganate (KMnO<sub>4</sub>) (BHD) is weighed and dissolved in 50 mL of Dw. The

solution is heated for 5 minutes to complete the dissolution and get rid of the remaining permanganate. The solution is cooled, filtered, and then diluted with the same solvent to 200 mL in a volumetric flask. The final solution obtained is titrated with a standard solution of sodium oxalate.

*Working Standard KMnO<sub>4</sub> Solution (1000 µg/mL):* Into a 100 mL volumetric flask, an appropriate volume of KMnO<sub>4</sub> stock solution is pipetted and then diluted to the mark with Dw. The prepared solution is kept in a dark bottle.

*Sodium Hydroxide Solution (1 M):* One ampoule of sodium hydroxide (at a concentration of 1 M/100 mL) (BHD) is diluted to a volume of one liter using Dw.

*Solution of indigo carmine Dye ( $5 \times 10^{-4}$  M):* 0.0232 g of indigo carmine dye is weighed and dissolved in a 100 mL Dw using a volumetric flask.

*N-bromosuccinimide Solution ( $2 \times 10^{-3}$  M):* 0.0356 g of N-bromosuccinimide (NBS) is dissolved in a portion volume of Dw and then completed to 100 mL with the same solvent in a volumetric flask.

Hydrochloric acid solution (1 M) is also prepared.

### 2.3. Analysis

#### 2.3.1. Method A (direct method)

Under ideal conditions, aliquots of the standard solution of PPH (100 µg/mL) covering the concentration range of 0.2 - 200 µg/mL are placed in a series of 25 mL volumetric flasks. A 2.0 mL of 1 M NaOH solution and 1.0 mL of KMnO<sub>4</sub> (1000 µg/mL) solution are added. The sample solutions are placed in a water bath whose temperature is fixed at 40°C for ten minutes, the solutions are then cooled to the laboratory temperature. The volume of each flask will be brought to the mark with Dw and the absorbance of each is measured at the wavelength of 610 nm against the blank solution.

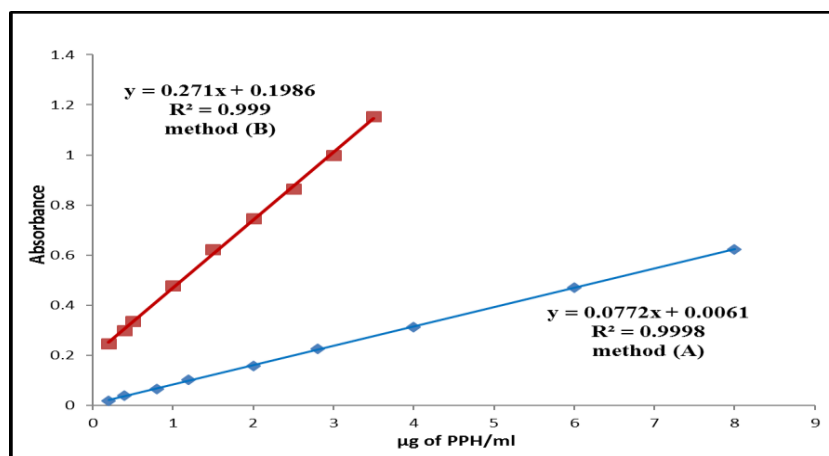
#### 2.3.2. Method B (indirect method)

To a series of 10 mL volumetric flasks, an increasing amounts 0.2-3.5 µg/mL of the standard PPH solution (20 µg/mL), 0.5 mL of 1 M hydrochloric acid and 1.0 mL NBS ( $2 \times 10^{-3}$  M) oxidizing agents are added. The solutions are mixed thoroughly and heated for 10 minutes at 50 °C using thermostatic water bath to complete the oxidation process of the PPH. After cooling to room temperature a 1.6 mL of indigo carmine dye ( $5 \times 10^{-4}$  M) is added. The solutions are mixed thoroughly and kept constant for 3.0 minutes. Finally, the volume is adjusted up to the mark with Dw and mixed well. The absorbance of each solution is recorded at 610.0 nm against corresponding reagent blank.

#### 2.3.3. Calibration graph

Under the optimum operating conditions, a linear calibration curve is obtained over the concentration ranges of 0.2 - 8.0 and 0.2 - 3.5 µg/mL of PPH with a molar absorption coefficients  $1.5722 \times 10^4$  and  $5.5191 \times 10^4$  L/mol.cm for methods A and B, respectively. The values of Sandell's sensitivity index are equal to 0.0129 µg.cm<sup>-2</sup> for method A and 0.00369

$\mu\text{g}\cdot\text{cm}^{-2}$  for method B. Fig.(1) explains the calibration curves of both suggested methods A and B.



**Figure 1:** Calibration graphs of PPH estimation by using methods A and B.

## 2.4. Essential Procedure for Assaying PPH in Drugs

### 2.4.1. For nasofen (1%) drop solution

Three containers of nasofen drops (each one contains 1% PPH) are mixed well to get a homogeneous solution. An aliquot volume, equivalent to 0.01 g of PPH, is transported to a 100 mL volumetric flask and with Dw diluted to the mark. Each mL of this solution contains 100  $\mu\text{g}$  of PPH. An aliquot of the diluted solution of the drop is then analyzed using the procedures designated in methods (A) and (B).

### 2.4.2. For injection PPH solution (500 $\mu\text{g}/10\text{ mL}$ ):

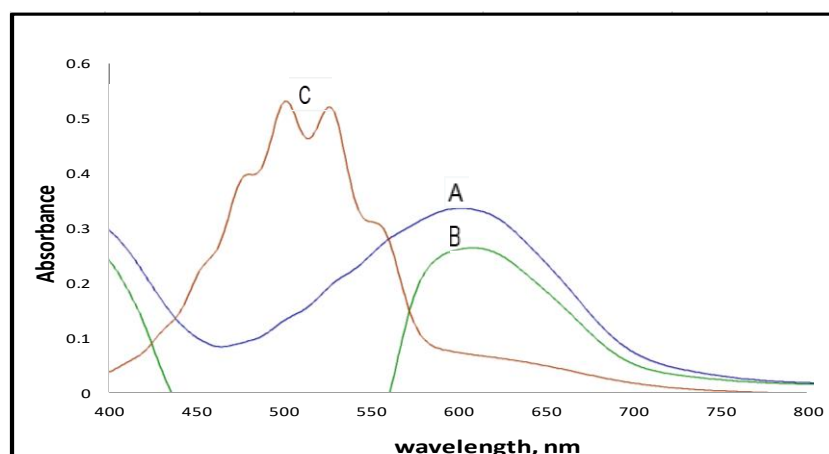
Three injections of PPH are mixed very well and transported to a washed dark bottle. Each mL of this

solution contains 50  $\mu\text{g}$  of PPH and an aliquot of the diluted drop is analyzed by using the procedures designated in (A) and (B) methods.

## 3. RESULTS AND DISCUSSION

### 3.1. Optimum Reaction Conditions

In method (A), phenylephrine is quantitatively oxidized with  $\text{KMnO}_4$  in the presence of alkaline solution of sodium hydroxide to yield a bluish-green color of manganate ( $\text{MnO}_4^{2-}$ ) which exhibits a peak with maximum absorption at the wavelength of 610 nm (Fig. 2).



**Figure 2:** Absorption spectra of 100  $\mu\text{g}/\text{mL}$  PPH Vs. (A) Dw, (B) blank solution, (C) blank solution Vs. Dw.

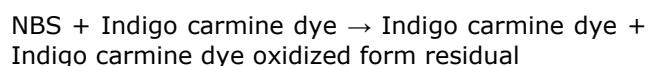
In method (B) the estimation of PPH includes two steps:

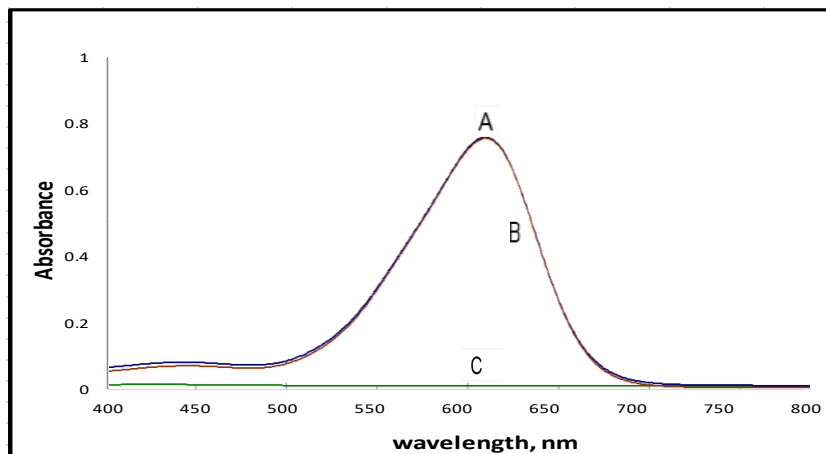
First step involves the oxidation of PPH by using an excess of oxidizing agent (NBS) in acidic medium.



Second step involves the residual and unreacted amount of NBS in bleaching the indigo carmine dye

and converting it to a colorless compound at the same media. The blue color of the residual indigo carmine dye is proportional to the PPH concentration that shows maximum absorption at 610 nm (Fig. 3).

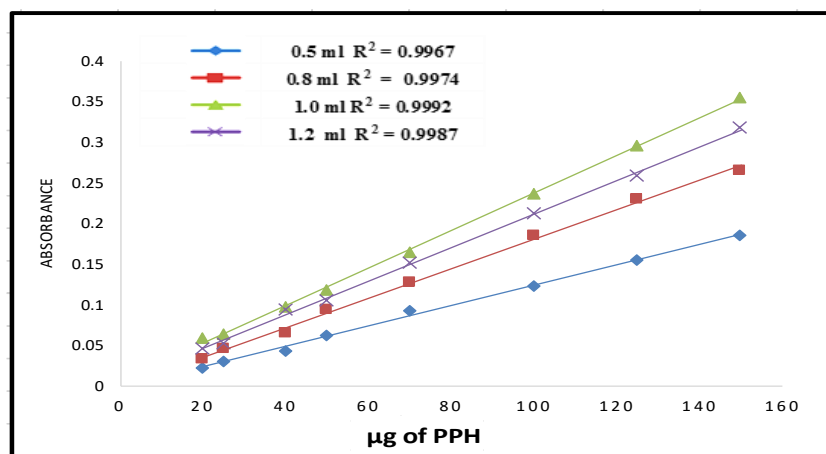




**Figure 3:** Absorption spectra of 20 µg/mL PPH Vs. (A) Dw, (B) blank solution, and (C) blank Vs. Dw.

The influence of diverse amounts (0.5-1.5 mL) of potassium permanganate on the absorbance of solutions containing increasing quantities of PPH (20–150 µg) in the presence of 1 M sodium hydroxide solution is studied. The experimental results in Figure 4 show

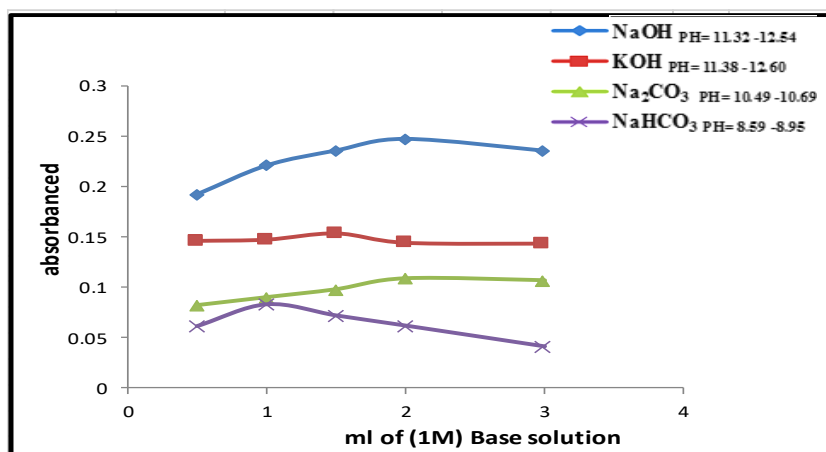
that 1.0 mL of potassium permanganate is the appropriate amount for the oxidation of PPH in aqueous medium with a good determination coefficient value ( $R^2=0.9992$ ). Therefore, 1 mL of potassium permanganate (1000 µg/mL) has been chosen as an optimum amount for the reaction.



**Figure 4:** Effect of the amount of potassium permanganate (1000 µg/mL) on absorbance.

The influence of various strong and weak alkaline solutions (1 M) on the improving the absorbance value of the resulting product is investigated. The results in Figure 5 reveal that the maximum sensitivity is obtained on using 1.8-2.5 mL of NaOH

solution. While  $\text{Na}_2\text{CO}_3$  and  $\text{NaHCO}_3$  display low sensitivity, which may due to pH variations. Therefore, 2.0 mL of 1 M NaOH solution has been relied upon for the subsequent experiments.

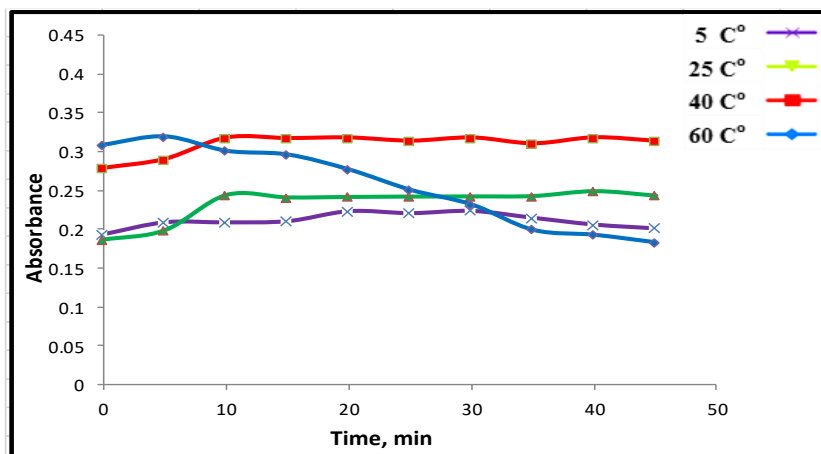


**Figure 5:** Influence of various alkaline solutions on absorbance.

The effect of addition sequence on the absorbance is also diagnosed. The experimental results indicated that the most favorable sequence of addition is PPH+NaOH+KMnO<sub>4</sub> due to its highest color intensity and development of maximum absorbance.

The effect of time that is required for the oxidation of PPH by KMnO<sub>4</sub> is investigated by following the color

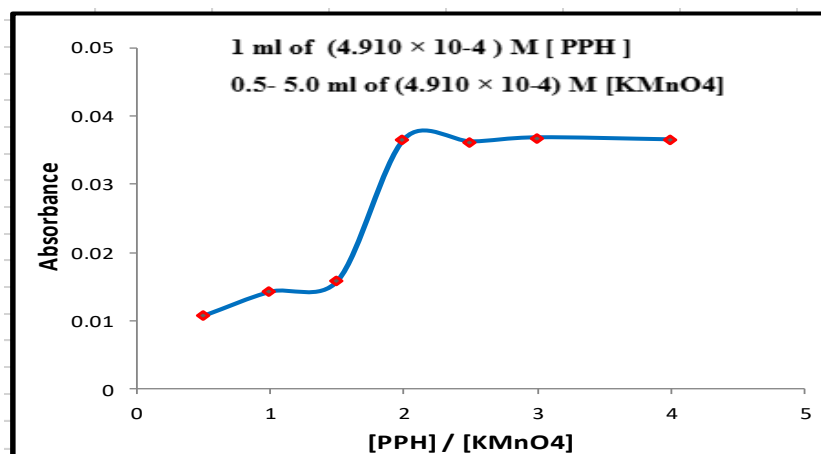
development of the product at different periods of time and temperatures up to 60 °C by using a water bath with thermostatic control. After completing the reaction at the fixed temperature and according to the approved method, the absorbance of the sample solution is measured at wavelength 610 nm against the reagent blank solution and the results are illustrated in Figure 6.



**Figure 6:** Effect of temperature and oxidation time on absorbance.

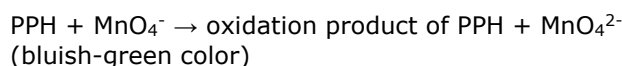
The results in Figure 6 indicate that the reaction of PPH with KMnO<sub>4</sub> in presence of NaOH solution is found to be complete in 10 min at temperature 40 °C. which gives a good sensitivity and stability. Therefore, these conditions are confirmed in the next experiments.

To find out the molar ratio of the oxidation reaction of PPH with KMnO<sub>4</sub> a molar ratio method is adopted (31), in which equal concentrations ( $4.910 \times 10^{-4}$  M) of the drug compound and the oxidizing agent KMnO<sub>4</sub> are used. The results in Figure 7 reveal that the ratio is 1:2 of PPH: KMnO<sub>4</sub>.



**Figure 7:** Molar ratio plot for reacting PPH with KMnO<sub>4</sub> under the optimum conditions.

Therefore, the chemical reaction equation of PPH with the oxidizing agent KMnO<sub>4</sub> in the basic medium can be written as:



### 3.2. Optimum Reaction Conditions of method (B)

All experiments are conducted in 10 mL volumetric flasks with 20 µg/mL of PPH and the measuring absorption for colored product is carried out at 610 nm.

### 3.3. Effect of the Amount of Indigo Carmine dye

In order to find the appropriate amount of the indigo carmine dye used in the reaction, the influence of several quantities from 0.2 to 3.0 mL of indigo carmine dye in acidic medium on the absorbance is investigated. The absorbance is measured at the improved wavelength 610 nm against the blank solution and the results are shown in Figure 8.

$$(\bar{x} - \mu) \frac{\sqrt{N}}{S}$$

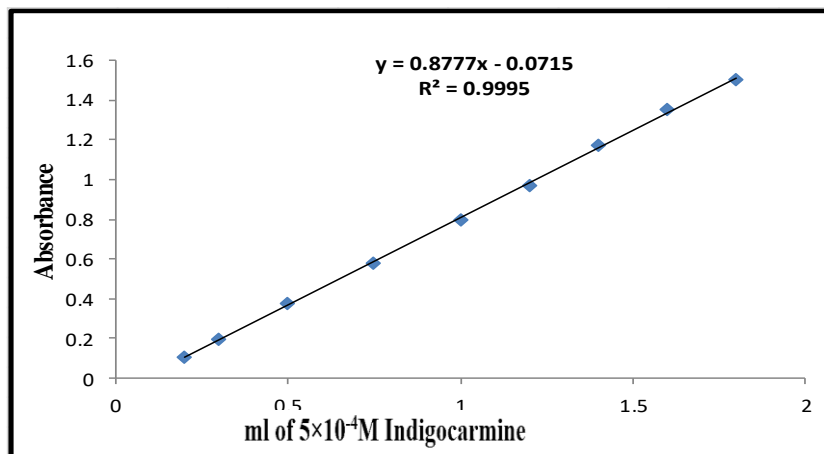


Figure 8: Standard curve of indigo carmine dye.

The results in Fig. 8 reveal that the linearity of the curve is continued to a volume of 1.8 mL of the dye, with determination coefficient of  $R^2 = 0.9995$ . Therefore, the amount 1.6 mL of the indigo carmine dye is chosen for the reaction.

**3.4. Influence of the Amount of NBS**

After the selecting the ideal amount of the indigo carmine dye, the effect of NBS amount required for the

bleaching  $5 \times 10^{-4}$  M indigo carmine dye is being carried out. Accordingly, the effect of different quantities (0.3 -1.7 mL) of NBS ( $2 \times 10^{-3}$  M) is studied in presence of 1 M HCl solution. The results in Figure 9 illustrate that a 1.0 mL of NBS is the optimal amount to reach almost complete dye color bleaching. Therefore, it has been proven and relied upon in subsequent experiments.

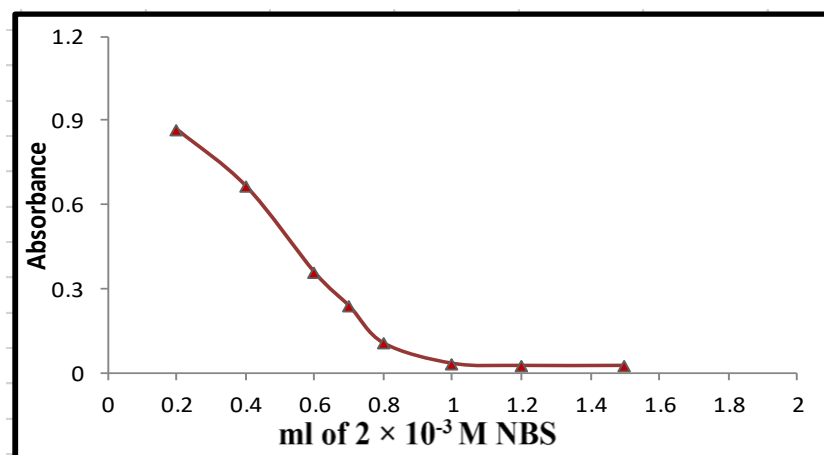


Figure 9: The effect of NBS amount on absorbance.

**3.5. Effect of the Type of Acid and its Amount**

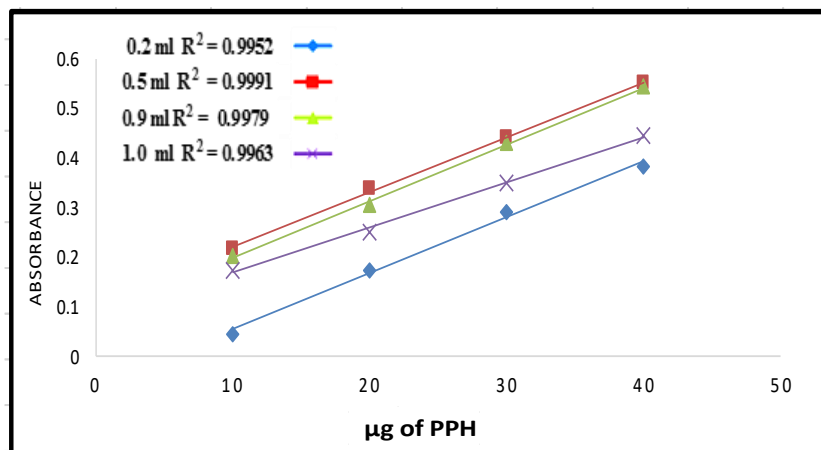
The influence of various strong and weak acids on the absorbance is studied. The results are listed in Table 1.

The results in Table 1 show that the hydrochloric acid solution is the best, so the effect of different concen-

trations of HCl solution on the absorbance is diagnosed. The results are explained in Figure 10 indicate that 0.5 mL of HCl acid (1 M) is selected as the ideal concentration because, it gives the best value of determination coefficient ( $R^2 = 0.9991$ ).

Table 1: Effect of the type of acid.

Type of acid (1 M)	HNO <sub>3</sub>	H <sub>2</sub> SO <sub>4</sub>	HCl	H <sub>3</sub> PO <sub>4</sub>	CH <sub>3</sub> COOH
Absorbance	0.0841	0.0623	<b>0.3225</b>	0.0694	0.1940
pH	1.98	2.04	<b>1.91</b>	2.34	2.82



**Figure 10:** Effect of the amount of 1 M HCl solution on absorbance.

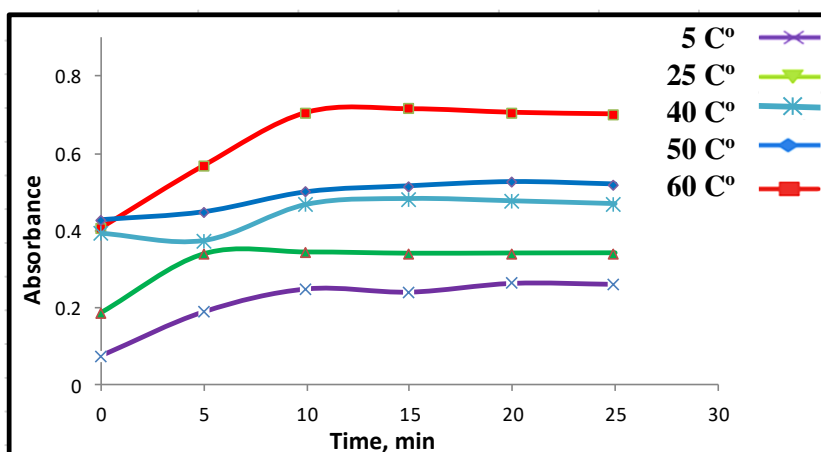
### 3.6. Effect of Oxidation Time and Temperature

It is necessary to investigate the ideal time and temperature that are required for the oxidation of PPH by NBS. The effect of different temperatures (5, 25, 40, 50 and 60 °C) on the absorbance using a water bath with thermostatic control is carried out (Figure 11).

The experimental results in Figure 11 show that the oxidation of PPH with NBS in the presence of HCl is

optimum at temperature of 50 °C after waiting for 10 minutes.

The influence of bleaching time of the indigo carmine dye by the unreacted amount of NBS is also investigated. The data listed in Table 2 show that 3.0 to 7.0 minutes are the optimum time which give the highest absorption intensity at 610 nm. Therefore, the time of 3.0 minutes is relied upon in the next experiments.



**Figure 11:** Effect of oxidation time and temperature on absorbance.

**Table 2:** Effect of bleaching time of indigo carmine dye on absorbance.

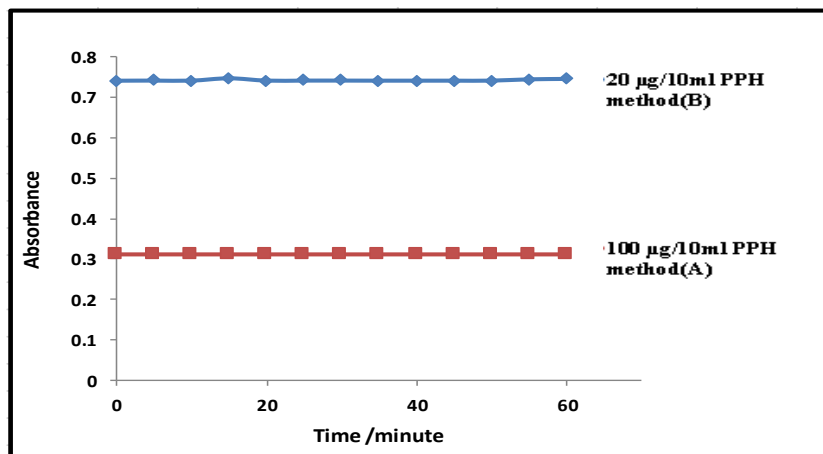
Time, min	2.0	3.0	5.0	7.0	10.0	15.0
<b>Absorbance</b>	0.7346	<b>0.7454</b>	0.7451	0.7450	0.7398	0.7386

The effect of different sequences on the intensity of absorption under the optimum experimental conditions is also tried. The experimental results indicated that the order of addition of (PPH + HCl + NBS + indigo carmine dye) is the best (A=0.7456).

### 3.7. Effect of Time on Color Development

In method (A), the absorption of the colored product of permanganate ion reaches the highest value after

10 minutes and remains stable for at least 40 minutes at room temperature. Whereas, in method (B) the blue color of the remaining indigo carmine dye after bleaching it with the excess of NBS stays stable for about an hour and there is no noticeable change in color and absorption during this period. The results for both methods are shown in Figure 12.



**Figure 12:** Effect of time on the development color for both methods (A) and (B).

### 3.8. Quantification

The limits of Beer's law, molar absorptivities, Sandell's sensitivities, accuracy (recovery %), and precisions (RSD) of the two methods (A) and (B) are evaluated. The linearity of both methods are also described by the equation of regression, as well as the corresponding determination coefficient ( $R^2$ ) for PPH

is calculated by the two recommended methods and represented excellent linearity. The limits of detection (LOD) and quantitation (LOQ) are found according to the ruling guidelines (32). The results are summarized in Table 3, which indicate that the proposed both methods are sensitive, precise, and accurate.

**Table 3:** Analytical data and optical characteristics of the proposed methods (A) and (B).

Parameters	Value	
	Method (A)	Method (B)
Beer's law range ( $\mu\text{g/mL}$ )	0.2 – 8.0	0.2 – 3.5
Molar absorptivity ( $\text{L/mol.cm}$ )	$1.5722 \times 10^4$	$5.5191 \times 10^4$
LOD ( $\mu\text{g/mL}$ )	0.00275	0.00077
LOQ ( $\mu\text{g/mL}$ )	0.00916	0.00258
Relative error range*	-0.1.066 to -0.32	-2.05 to -0.73
Recovery (%) range*	99.68 to 98.93	97.95 to 99.26
RSD*	0.0286 to 0.1783	0.0114 to 0.00846
Determination coefficient ( $R^2$ )	0.9998	0.9990
Slope (a) <sup>#</sup>	0.0772	0.271
Intercept (b) <sup>#</sup>	0.0061	0.1986
Sandell's sensitivity	0.0129	0.00369

\*Average of five estimations, # Regression equation ( $X = b + ac$ ), where c is (PPH) in  $\mu\text{g/mL}$ .

### 3.9. Application

Both methods (A) and (B) are applied to estimate PPH in its pharmaceutical preparations (injection and drop) for four different concentrations 20, 100, and 150  $\mu\text{g}$  (method A) and 10, 20, and 30  $\mu\text{g}$  of PPH (methods B). The results listed in Table 4 reveal that the proposed procedures (A) and (B) are in good agreement and with the declared content.

To evaluate the results of the proposed methods (A) and (B) a t-test has been carried out. The results of the t-test listed in Table 4 reveal that the values of t-exp. are less than the t-tabulated value at 95% confidence level and for four degrees of freedom ( $N = 4$ ) (33). This means that the difference is statistically not significant, which confirms the success of the two proposed methods for assaying PPH in its drugs.



**Table 4:** Analysis of PPH in pharmaceuticals preparations for methods (A) and (B).

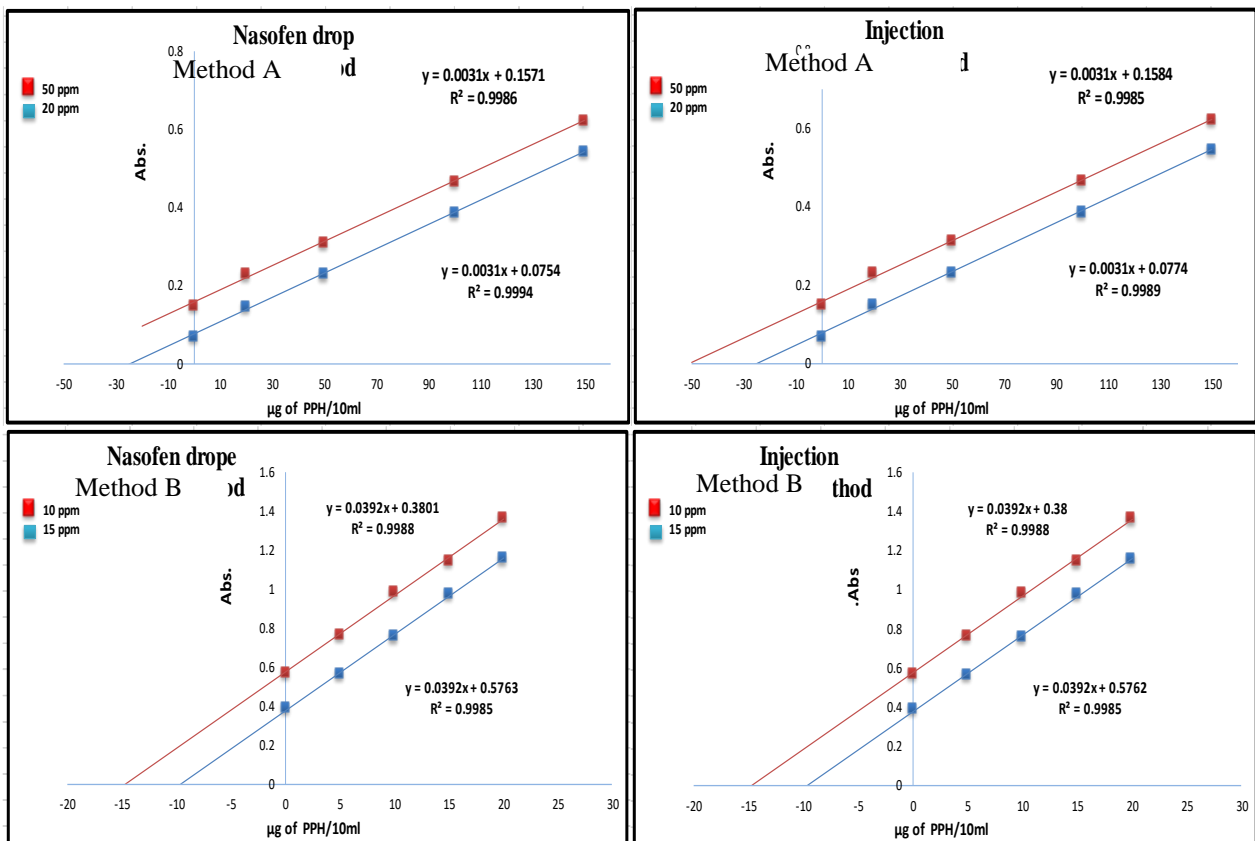
Drug Form	Certified Value	Method (A)			Method (B)		
		Found (µg)	Rec.(%) ± RSD (N=5)	Measured value	Found (µg)	Rec.(%) ± RSD (N=5)	Measured value
Phenylephrine injection (France)	500 µg PPH/10 mL	19.69	98.91± 0.410 t=1.001	494.55 µg	9.51	97.72 ± 0.624 t=0.936	488.6 µg
		99.84	98.397± 0.533 t=1.010	492 µg	18.68	98.77 ± 0.481 t=2.450	493.85 µg
		151.21	99.32 ± 0.941 t=2.451	496.6 µg	31.31	99.39 ± 0.441 t=1.633	499.65 µg
Nasofen drop (Pioneer-Iraq)	1.0%	19.82	98.94 ± 0.562 t=1.633	0.989%	9.37	98.34 ± 1.320 t=1.512	0.983%
		99.61	98.29± 0.607 t=1.643	0.983%	17.73	98.29± 0.592 t=1.001	0.983%
		151.27	97.97 ± 0.273 t=1.071	0.979%	28.56	98.74± 0.377 t=2.449	0.987%

$t \pm = (\bar{x} - \mu) \frac{\sqrt{N}}{s}$ , <sup>a</sup>Tabulated "t" value at 95% confidence level is equal to 2.776.

**3.10. Evolution of the Proposed Methods**

To prove the efficiency and credibility of the two proposed methods (A) and (B) in the estimation of PPH and to ensure that they are free from the interference of additives, a standard additions method is applied.

The results listed in Table 5 and shown in Figure 13 indicate that there is a high agreement between the standard additions method and the proposed methods (A and B) for the determination of PPH in its pharmaceutical preparations.



**Figure 13:** Standard addition curves for the estimation of PPH in pharmaceutical preparations by using methods (A) and (B).

**Table 5:** The results of standard addition methods for analysis of PPH in its drugs.

Drug	Certified Value	Method (A)		Average of recovery (%)	Average of measured value
		PPH Present ( $\mu\text{g}$ )	PPH Measured ( $\mu\text{g}$ )		
Phenylephrine injection (France)	500 $\mu\text{g}$ PPH/mL	20	20.06	102.59	512.95 $\mu\text{g}$
		50	51.09		
Nasofen drop (Pioneer-Iraq)	1.0%	20	20.8	102.6	1.026 %
		50	50.6		
Method (B)					
Phenylephrine injection (France)	500 $\mu\text{g}$ PPH/mL	10	9.69	97.42	487.08 $\mu\text{g}$
		15	14.69		
Nasofen drop (Pioneer-Iraq)	1.0%	10	9.69	97.45	0.975 %
		15	14.70		

#### 4. CONCLUSION

Two simple spectrophotometric methods (direct and indirect) are developed for estimating PPH in the bulk and in the pharmaceutical formulations through the oxidation-reduction reactions. The two suggested methods have many advantages of being accurate, sensitive, and convenient for routine analysis in control laboratories. As well as the resulting colored products of both methods are characterized by high stability and did not exhibit a noticeable change in absorption for at least 60.0 minutes. Beer's law of the both methods are linear in the concentration ranges 0.2–8.0 and 0.2–3.5  $\mu\text{g}/\text{mL}$  for method A and method B, respectively. The values of molar absorptivity of methods A and B are  $1.5722 \times 10^4$  and  $5.5191 \times 10^4$  L/mol.cm, respectively. Both methods A and B have been successfully applied for analysis of PPH in drop and injection drugs with an excellent recoveries from 97.97% to 99.32% for method A and 97.72% to 99.39% for method B. The future works will include an attempt to establish an analytical procedure for simultaneous estimation of PPH and tetracycline in the pharmaceutical formulations by using second derivative spectrophotometry.

#### 5. CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

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