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Research Article

Sensitive Spectrophotometric Determination of Phenylephrine Hydrochloride Based on Diazotized 2,4,6-Trichloroaniline

[1,] Skala S. Salim [2,] Nabil A. Fakhre
[1,] Department of Chemistry, College of Education, Salahaddin University-Erbil, Erbil, Kurdistan Region, Iraq
[2,] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Tishk International University, Erbil, Kurdistan Region, Iraq

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Abstract

The study introduces 2,4,6-trichloroaniline as a novel coupling reagent for sensitive spectrophotometric determination of phenylephrine hydrochloride. The analytical method is based on the interaction of diazotized 2,4,6-trichloroaniline and phenylephrine hydrochloride under basic conditions to form a stable chromogenic compound. The compound exhibits strong yellow color and has a maximum absorbance wavelength at 410 nm, thereby enabling precise quantitative analysis. The method parameters such as temperature, reaction time, and reagent concentrations were carefully optimized to ensure improving sensitivity, high reproducibility and robustness of the procedure. Beer's law was found to be obeyed within the linear range of (0.5–12.0 µg/mL), limit of quantification (0.1289 µg/mL), limit of detection (0.0425 µg/mL), and (R^2 = 0.9997). The molar absorptivity coefficient was (1.58×10^4 $L.mol^{-1}\cdot cm^{-1}$), which indicates the sensitivity of the method. Up to 60 minutes, the color of the product remained stable, and there was no significant change in the absorbance of the colored compound. For indicating the precision of the method, the results show the percentage of relative standard deviation of 0.16%, and an accuracy with an error percentage was 99.86% based on multi replicate analysis (n = 3). When the suggested method and the High-Performance Liquid Chromatography standard method were compared, the calculated t-value and F-test which were less than the critical t-value and F-value, indicating that there was no significant difference between the two methods. Overall, this method provides an efficient, cost-effective, and reliable means for the routine analysis of phenylephrine hydrochloride in pharmaceutical formulations.

Corresponding Author E-mail: Skala.saleem@su.edu.krd , nabil.fakhre@su.edu.krd
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1. Introduction

phenylephrine hydrochloride (PPH) belongs to the class of sympathomimetic drugs [1]. It is an effective vasopressor when administered parenterally or orally and acts primarily as a vasoconstrictor. Phenylephrine exerts its pharmacological action by selectively stimulating α_1-adrenergic receptors, resulting in vasoconstriction with minimal reflex cardiac stimulation. In ophthalmic applications, phenylephrine produces mydriasis by relaxing the iris sphincter muscle and contracting the radial muscle fibers, making it useful in diagnostic and therapeutic procedures [2]. In addition, nasal formulations of phenylephrine hydrochloride are commonly used to relieve symptoms such as rhinorrhea, sneezing, and nasal and pharyngeal pruritus [3]. Although rarely, phenylephrine is also employed clinically as a vasopressor to elevate blood pressure [4]. (Figure 1.1) shows the chemical structure of PPH.

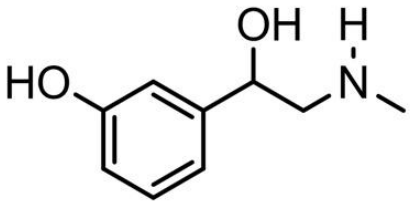


Figure 1.1 : The chemical structure of phenylephrine hydrochloride (PPH).

The diazotization–coupling reaction is one of the most widely used methods in pharmaceutical analysis. For the determination of phenylephrine hydrochloride (PPH), an azo-coupling reaction between the drug and a suitable reagent is commonly applied, resulting in the formation of a colored azo dye whose absorbance can be measured spectrophotometrically in the visible region [5]. Several analytical techniques have been reported for the determination of phenylephrine hydrochloride, including chemometric-assisted spectrophotometry, reversed-phase high-performance liquid chromatography (RP-HPLC) [6], dual-wavelength spectrophotometric methods for the simultaneous estimation of PPH in bulk and pharmaceutical dosage forms [7], conductometric titration [8], voltammetry [9], derivative spectrophotometry [10], high-performance thin-layer chromatography (HPTLC) [11], and RP-HPLC coupled with a photodiode array detector (RP-HPLC-PDA) [12]. Among these techniques, UV–Visible spectrophotometry is preferred for routine pharmaceutical analysis due to its simplicity, low cost, and the availability of basic instrumentation in most laboratories. In addition, it has been successfully applied for the determination of PPH in pharmaceutical preparations and biological fluids. Several spectrophotometric methods reported in the literature are based on oxidative or diazotization–coupling reactions of PPH with various reagents, such as 4-aminoantipyrine in the presence of potassium ferricyanide [13], N,N-dimethyl-p-phenylenediamine with $FeCl_3$ in alkaline medium [14], N,N-dimethyl-p-phenylenediamine with sodium persulfate [15], as well as diazotized p-nitroaniline [16], 2-aminobenzothiazole [17], sulfacetamide sodium [18], 2,4-dinitroaniline [19], clonazepam [5], and 2-aminothiazole [20] as coupling reagents. We used 2,4,6-trichloroaniline as a coupling reagent offers enhanced sensitivity in azo dye formation due to the presence of the three chloro (-Cl) groups at positions 2,4 and 6,

which increase molar absorptivity , surpassing many simpler aniline derivatives used previously. These chloro substituents also contribute to the chemical stability of the azo dye, making it highly resistant to degradation from light, temperature fluctuations, and pH change, thus ensuring consistent analytical performance [21]. Additionally, the coupling reaction with this reagent can be conducted at room temperature (25 ± ℃), eliminating the need for external heating or cooling and thereby conserving energy in line with green chemistry principles. Compared to other coupling reagents like 4-bromoaniline or 2,4-dinitroaniline that have shown good sensitivity and stability in phenylephrine hydrochloride determination, 2,4,6-trichloroaniline is expected to provide comparable or improved performance due to its electron-withdrawing chlorine groups enhancing azo dye properties. This combination of enhanced sensitivity, stability, and sustainable reaction conditions makes 2,4,6-trichloroaniline a promising reagent for spectrophotometric assays of phenylephrine hydrochloride.

This study presents a sensitive and reliable spectrophotometric method for the determination of phenylephrine hydrochloride (PPH). The proposed method is based on a diazotization–coupling reaction between PPH and diazotized 2,4,6-trichloroaniline, producing a yellow, water-soluble azo dye. The novelty of this study lies in the use of 2,4,6-trichloroaniline as the coupling agent. The formed chromophore exhibits a maximum absorbance at 410 nm in an alkaline medium. Furthermore, the experimental conditions were optimized to ensure accurate, precise, and efficient determination of PPH in pharmaceutical formulations.

2. Experimental

2.1 Instruments

All spectral and absorbance measurements were carried out using a Shimadzu UV-1800 double-beam UV–Visible scanning spectrophotometer equipped with a 1.0 cm quartz cell. An analytical balance (KERN ABS) was used for weighing.

2.2 Chemicals

All reagents used were of analytical grade. Standard phenylephrine hydrochloride (purity 99%) was purchased from Awa medica Company. 2,4,6-Trichloroaniline (98%) was obtained from Fluka AG, sodium hydroxide (97%) from Scharlau, hydrochloric acid (37%, Merck), sodium nitrite (99.50%) from Riedel-de Haën AG, and ethanol (99.9%) from Merck.

2.3 Preparation of Solutions

A stock solution of phenylephrine hydrochloride (100 µg/mL) was prepared by accurately dissolving 0.0100 g of pure phenylephrine hydrochloride in distilled water and diluting to 100 mL in a volumetric flask, as reported previously [17]. The solution was stored in a dark-colored container away from direct sunlight [22].

A 0.1% (w/v) solution of 2,4,6-trichloroaniline was prepared by dissolving 0.1000 g of the reagent in ethanol and diluting to 100 mL with the same solvent.

Sodium hydroxide solution (1.0 N) was prepared by dissolving 4.0000 g of NaOH in distilled water and diluting to 100 mL.

Hydrochloric acid solution (1.0 M) was prepared by diluting 8.2800 mL of concentrated hydrochloric acid with distilled water in a 100 mL volumetric flask.

Sodium nitrite solution (0.5% w/v) was prepared by dissolving 0.5000 g of sodium nitrite in distilled water and diluting to 100 mL.

Nasal drop solutions were prepared by transferring 1.0 mL of Nasofen nasal drops (1.0%) obtained from Pioneer Company into a 100 mL volumetric flask and diluting to the mark with distilled water [22]. The same procedure was applied for the preparation of Nazafrin nasal drops (1.0%) obtained from Safa Company.

2.4 Preparation of the Azo Dye

In a series of 10.0 mL volumetric flasks, 1.3 mL of 0.1% 2,4,6-trichloroaniline solution was mixed with 1.0 mL of hydrochloric acid (1.0 M) and 0.5 mL of sodium nitrite solution (0.5%) at room temperature. An appropriate aliquot of phenylephrine hydrochloride solution corresponding to a concentration range of 0.5-12.0 µg/mL was then added. Subsequently, 1.3 mL of sodium hydroxide solution (1.0 N) was added to each flask, and the mixtures were diluted to volume with ethanol. The absorbance of the resulting yellow azo dye was measured at 410 nm against a reagent blank.

3. Results and Discussion

3.1 The Principal of the Method

The reaction mechanism of the proposed method involves a diazotization-coupling process proceeding in two successive steps, resulting in the formation of a highly colored yellow azo dye, as illustrated in (Figure 3.1) in the first step, 2,4,6-trichloroaniline undergoes diazotization in the presence of hydrochloric acid and nitrite ions generate 2,4,6-trichlorophenyl diazonium chloride. In the second step, this electrophilic diazonium species undergoes an electrophilic aromatic substitution reaction with the electron-rich aromatic ring of phenylephrine hydrochloride under alkaline conditions, yielding a yellow azo chromophore. The formation of this conjugated azo system is responsible for the observed maximum absorbance at 410 nm, providing a sensitive spectrophotometric signal.

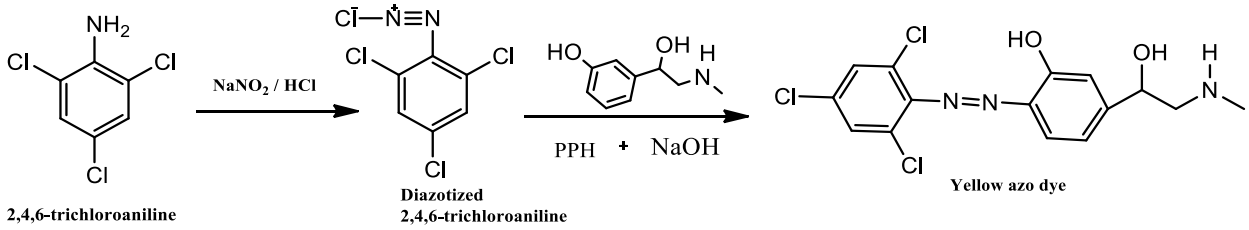


Figure 3.1 The schematic mechanism of the proposed reaction.

3.2 Optimization

In order to optimize the experimental conditions for the quantitative determination of phenylephrine hydrochloride, the present study is based on a diazotization-coupling reaction with 2,4,6-trichloroaniline. Various analytical parameters were investigated, including the concentration and volume of 0.1% 2,4,6-trichloroaniline, the type and concentration of acid and base, as well as the concentrations and volumes of hydrochloric acid, sodium nitrite, and sodium hydroxide. In addition, the stability of the yellow azo dye formed during the reaction was examined, since these parameters significantly affect the color intensity of the dye. The formed azo dye must exhibit sufficient stability to ensure accurate and reliable quantification.

3.2.1 Absorption Spectra

Phenylephrine hydrochloride was reacted with diazotized 2,4,6-trichloroaniline in a basic medium to produce a yellow azo dye.

The color of the dye stabilized after 15 minutes and exhibited maximum absorption at 410 nm. The absorption spectrum of the azo dye, measured against a reagent blank, is shown in Figure 3.2(b), while Figure 3.2(a) shows the absorption spectrum of the blank using distilled water as reference.

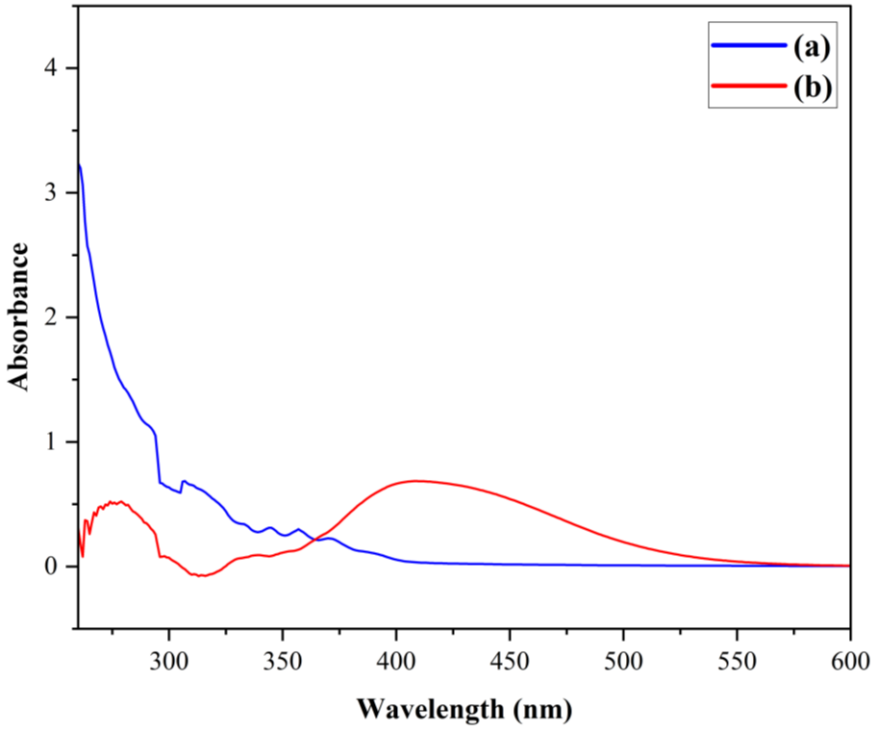


Figure 3.2. (B) Absorption spectrum of the azo dye at a concentration of 10 µg/mL recorded against a blank. **(A)** Absorption spectrum of the blank using distilled water as reference.

3.2.2 Effect of the Amount of 2,4,6-Trichloroaniline

The effect of the volume of 0.1% 2,4,6-trichloroaniline reagent on the color intensity of the formed azo dye was investigated. The volumes tested ranged from 0.3 to 1.5 mL. As illustrated in Figure 3.3, the results indicate that 1.3 mL of 2,4,6-trichloroaniline is sufficient to achieve the maximum absorbance of the azo dye.

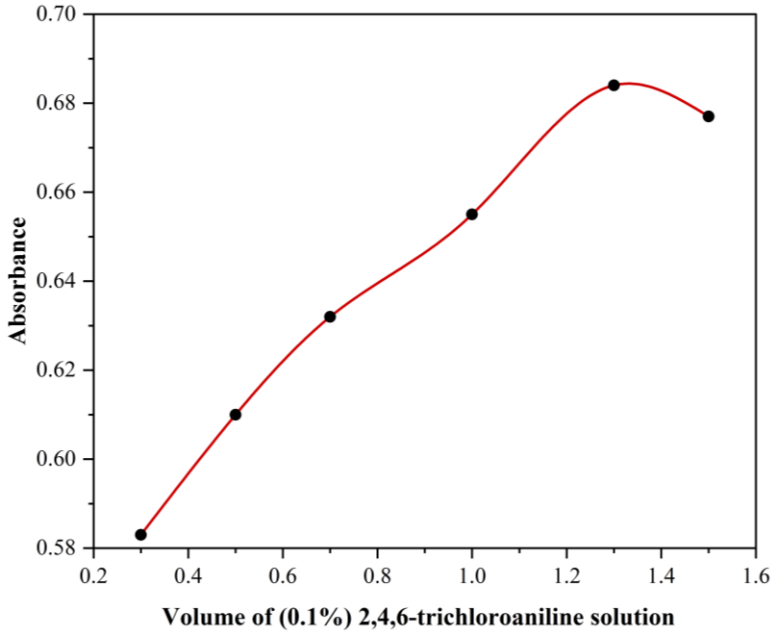
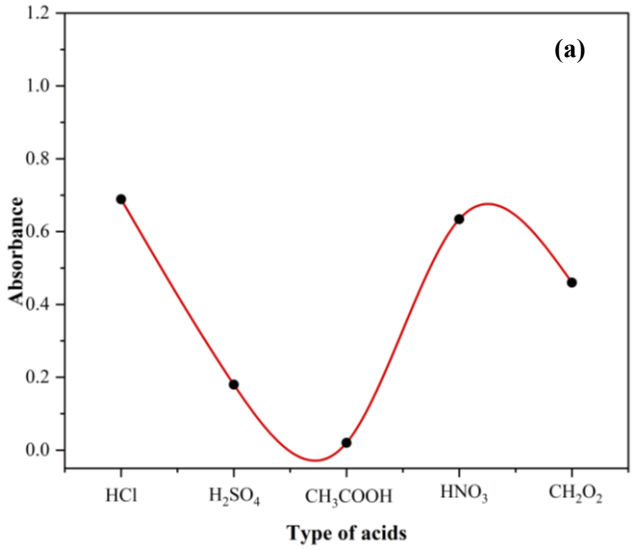


Figure 3.3. Effect of different volumes of 0.1% 2,4,6-trichloroaniline on the absorbance of phenylephrine hydrochloride (PPH).

3.2.3 Effect of Acids

The effect of various acids (1.0 M) on the diazotization reaction was studied, including HCl, H_2SO_4, CH_3COOH, HNO_3, and CH_2O_2. As shown in Figure 3.4a, hydrochloric acid gave the highest absorbance and provided the greatest stability of the formed azo dye.



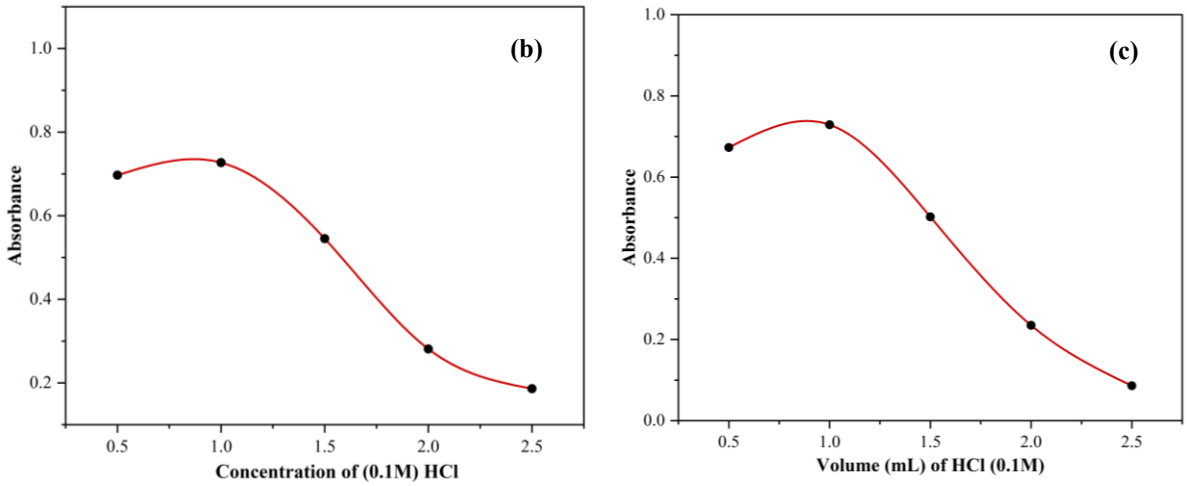


Figure 3.4. (A) Effect of different types of acids (1.0 M, 1.0 mL) on the absorbance of phenylephrine hydrochloride (PPH). (B) Effect of varying concentrations of HCl (1.0 M) on the absorbance of PPH. (C) Effect of the volume of HCl (1.0 M) on the absorbance of PPH.

Due to its superior absorbance and stability in the diazotization process, hydrochloric acid (HCl) was found to be the most effective acid among those tested. In contrast, sulfuric acid (H_2SO_4) and nitric acid (HNO_3) can disrupt the reaction due to their dehydrating and oxidizing properties, respectively. Weak acids such as acetic acid (CH_3COOH) and formic acid (HCOOH), because of their limited dissociation, are unable to provide sufficiently acidic conditions for optimal reaction progress [23].

The effect of HCl concentration on the absorbance of the azo dye was investigated over a range of 0.5–2.5 M. As shown in Figure 3.4b, 1.0 M HCl provided the highest absorbance. The influence of acid volume was also studied, and Figure 3.4c shows that 1.0 mL of 1.0 M HCl gave the maximum absorbance. The absorbance increased with increasing acid volume up to this optimum, but decreased sharply at higher volumes. This decrease is attributed to the formation of diazonium salts ($Ar\text{-}NH_3^+Cl^-$) from the diazonium ion, reducing the effective concentration of reactive species responsible for the dye's absorbance [24].

3.2.4 Effect of Sodium Nitrite (% $NaNO_2$)

Sodium nitrite solutions of the same volume but varying concentrations were used to study their effect on the intensity of the azo dye. As shown in Figure 3.5a, a 0.5% sodium nitrite solution gave the maximum absorbance. The effect of different volumes (0.1–2.0 mL) of 0.5% sodium nitrite on the azo dye intensity was also investigated. Figure 3.5b shows that 0.5 mL of 0.5% sodium nitrite solution is sufficient to achieve maximum absorbance. This volume was used in subsequent experiments for the diazotization of the amino group [25].

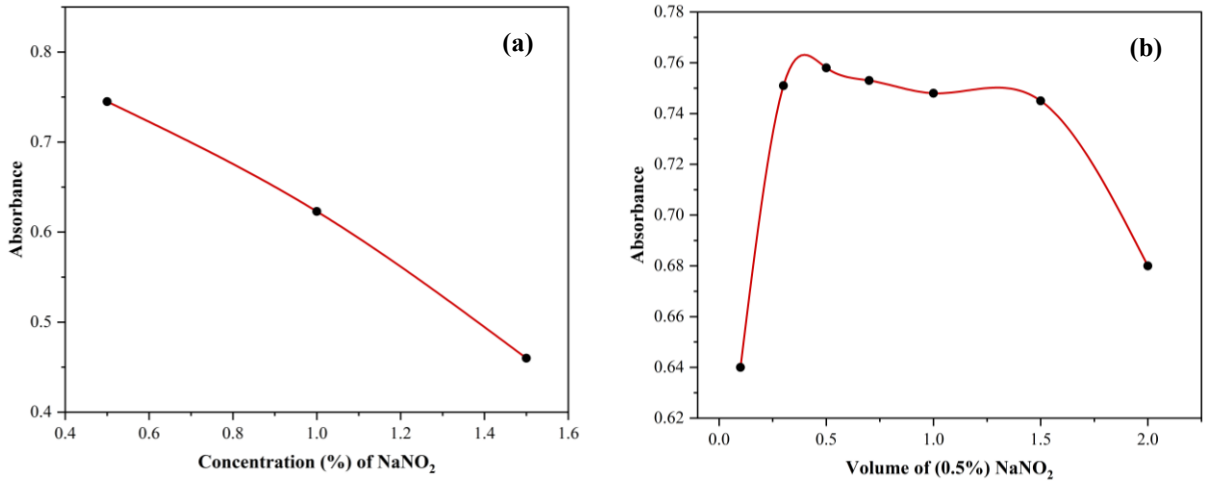


Figure 3.5. (A) Effect of different concentrations (%) of $NaNO_2$ (1.0 mL) on the absorbance of phenylephrine hydrochloride (PPH). (B) Effect of different volumes of 0.5% $NaNO_2$ on the absorbance of PPH.

3.2.5 Effect of Bases

The effect of various strong and weak bases, all at the same concentration, including sodium hydroxide, potassium hydroxide, sodium carbonate, and ammonia solution, on the absorbance of the azo compound was compared. The chromophore reached its optimal structural conformation after a 15-minute incubation period. As shown in Figure 3.6a, 1.0 N sodium hydroxide provided the highest absorbance, as well as the best sensitivity and stability, indicating that strongly basic conditions are necessary for the formation of the colored dye. The effect of sodium hydroxide volume on the azo dye formation was also investigated. Figure 3.6b shows that 1.3 mL of 1.0 N sodium hydroxide gives the highest absorbance and was used in subsequent experiments to ensure optimal sensitivity [17].

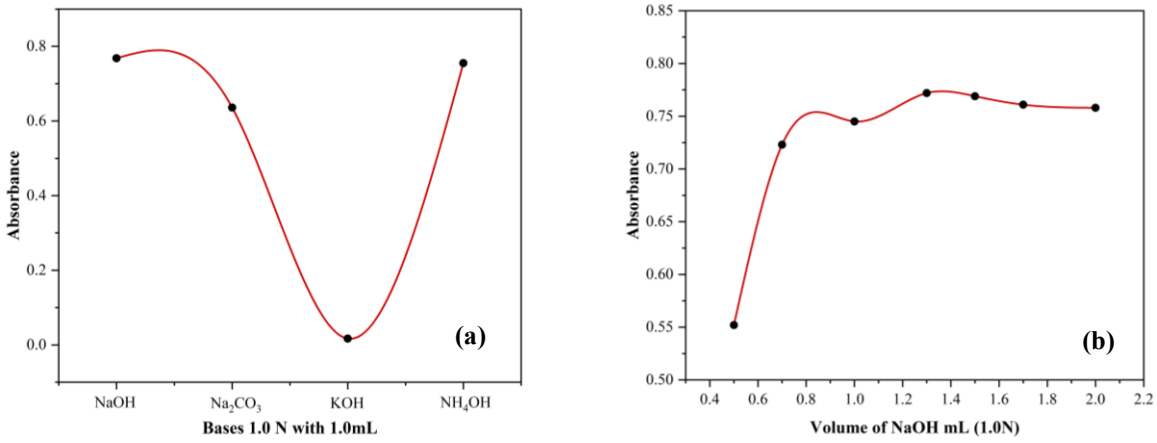


Figure 3.6. (A) Effect of different types of 1.0 N bases (1.0 mL) on the absorbance of phenylephrine hydrochloride (PPH). **(B)** Effect of different volumes of 1.0 N NaOH on the absorbance of PPH.

3.2.6 Stability of the Yellow Azo Dye Product

The yellow-orange azo dye is formed immediately upon the addition of the base and, after dilution, reaches its optimal structure after 15 minutes of incubation. As shown in Figure 3.7, the dye remains stable for approximately 60 minutes, with no significant changes in absorbance observed during this period.

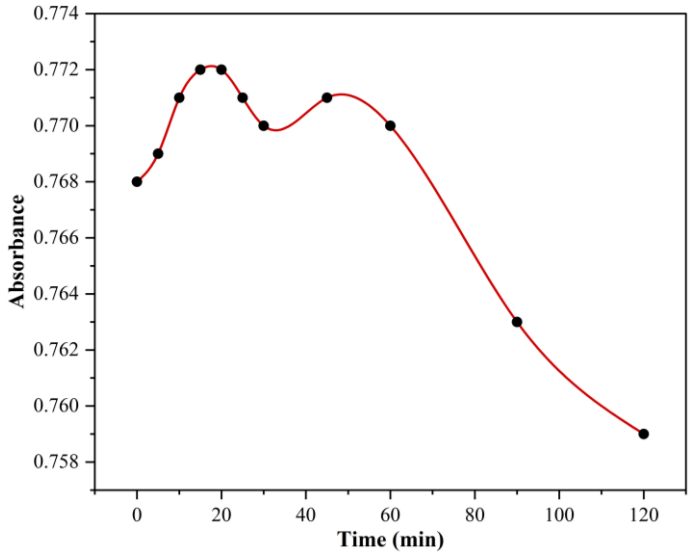


Figure 3.7. Effect of time on the stability of the yellow-orange azo dye.

3.3Calibration Curve

Under the described experimental conditions, 10 mL volumetric flasks were used. To each flask, 1.3 mL of 0.1% 2,4,6-trichloroaniline solution was added, followed by the addition of 1.0 mL of 1.0 M hydrochloric acid and 0.5 mL of 0.5% sodium nitrite at room temperature with gentle shaking. Aliquots of phenylephrine hydrochloride (PPH) at concentrations from 0.5 to 12.0 µg/mL were then added to each flask. Subsequently, 1.3 mL of 1.0 N sodium hydroxide was added, and the solutions were diluted to the mark with ethanol.

The absorbance at 410 nm was measured against a reagent blank, and a calibration curve was constructed (Figure 3.8). The Beer-Lambert law was obeyed over the concentration range of 0.5–12.0 µg/mL. The apparent molar absorptivity of phenylephrine hydrochloride was calculated to be 1.58×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$.

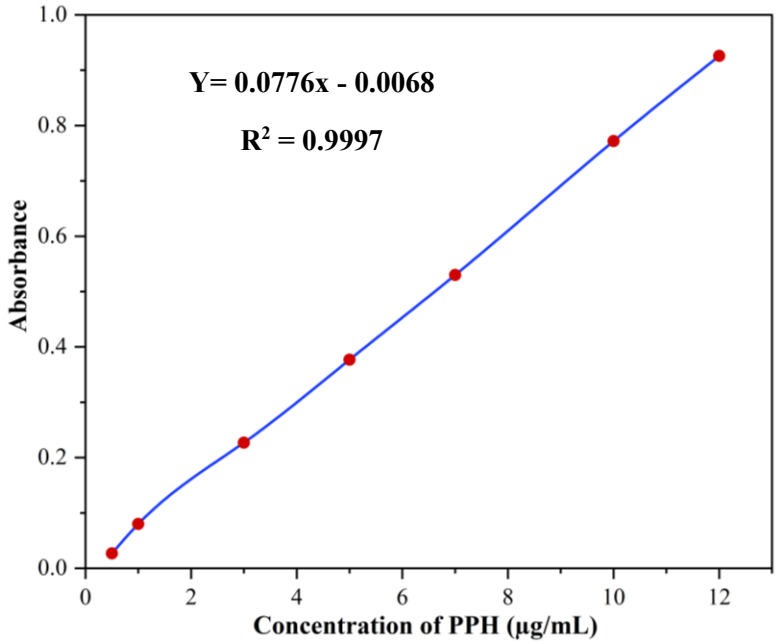


Figure 3.8 Calibration curve of phenylephrine hydrochloride (PPH) showing the relationship between concentration (0.5–12.0 µg/mL) and absorbance.

3.3.1 Analytical Features of the Spectrophotometric Technique

A series of standard solutions was prepared under the optimized reaction conditions, and a calibration curve was constructed (Figure 3.8). All measurements were performed in triplicate to verify the linearity of the method. The results, presented in Table 3.1, include the molar absorptivity (ε), correlation coefficient (R^2), slope (a), intercept (b), limit of detection (LOD), limit of quantification (LOQ), and Sandell's index.

The molar absorptivity (ε) of the colored product was calculated from the slope of the calibration curve using the following (Eq.1), Where M.wt is the molecular weight of phenylephrine hydrochloride (203.67 g/mol) with a slope of 0.0776 L/mg, was found to be 1.58×10^4 $L.mol^{-1}.cm^{-1}$ (for a 1 cm path length), confirming the good sensitivity of the proposed method. = Slope (L/mol^{-1}) = Slope (L/mg^{-1}) × $M.wt$ ×1000 (1)

= 0.0776 L/mg^{-1} × 203.67 ×1000 = 1.58×10^4 $L.mol^{-1}.cm^{-1}$

The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the (Eq. 2 and 3) respectively [26]. Where σ is the standard deviation of the blank response (n = 3) and S is the of the calibration curve. Based on triplicate blank measurements the σ was calculated as 0.001. Using S = 0.0776 L/mg^{-1}, the LOD and LOQ were found to be 0.0425 µg/mL and 0.1289 µg/mL, respectively, indicating high sensitivity of the proposed method.

$$LOD = \frac{3.3 \times \sigma}{S} = \frac{3.3 \times 0.001}{0.0776} = 0.0425 \quad (2)$$

$$LOQ = \frac{10 \times \sigma}{S} = \frac{10 \times 0.001}{0.0776} = 0.1289 \quad (3)$$

Table 3.1. Analytical characteristics of the developed spectrophotometric method for the determination of phenylephrine hydrochloride (PPH).

Parameter	Values
λ_{max} (nm)	410
Linear range (µg/mL)	0.5 -12
Regression equation	Y= 0.0776x - 0.0068
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.58×10^4
Correlation coefficient (R^2)	0.9997
LOD	0.0425
LOQ	0.1289
Sandell's index (µg/cm^2)	0.00746

3.3.2 Precision and Accuracy

The validity and reproducibility of the developed analytical method were evaluated by performing triplicate analyses of phenylephrine at three different concentration levels using the proposed spectrophotometric technique. The relative standard deviation (% RSD), recovery (%R), and relative error (%Error) values are presented in Table 3.2. The results demonstrate that the method is highly precise and accurate, confirming its suitability for the precise quantitative determination of phenylephrine hydrochloride.

Table 3.2. Accuracy and precision of phenylephrine hydrochloride (PPH) determination using the proposed spectrophotometric method.

Concentration of PPH	% Recovery*	% Error	% RSD
3.0	99.9%	±0.29%	0.21%
7.0	99.86%	±0.18%	0.15%
12.0	99.82%	±0.07%	0.14%

(*); average of (n=3) replication.

3.4 Interferences

The allowable limit of interference was defined as an absorbance change not exceeding ±5.0%. The effect of common preservatives used in pharmaceutical formulations, including methyl paraben, propyl paraben, and benzalkonium chloride, was carefully evaluated to assess the accuracy, specificity, and ro-

bustness of the method. Complete experimental protocols were carried out accordingly. As shown in Table 3.3, the results indicate that these additives and excipients did not interfere with the analytical measurement, confirming the selectivity and reliability of the proposed method.

Table 3.3. Effect of common excipients and additives on the determination of phenylephrine hydrochloride (PPH) in the sample.

Preservatives	Conc of PPH, 10µg/mL (Found)	% interferences
Methyl paraben + Propyl paraben	10	0%
Benzalkonium chloride	9.95	-0.5%

3.5 Stoichiometry of the Resulting Product

The stoichiometry of the reaction between phenylephrine hydrochloride (PPH) and 2,4,6-trichloroaniline (diazonium salt) was investigated using the mole ratio method. In this experiment, added 1.0 mL of 2,4,6-trichloroaniline diazonium salt solution (4.91×10^{-4} mol/L). to a series of 10 mL volumetric flasks was mixed with 1.0 mL of hydrochloric acid (1.0 M) and 0.5 mL of sodium nitrite solution (0.5%) at room temperature. and 0.25-3.0 mL of phenylephrine hydrochloride solution (4.91×10^{-4} mol/L) was added. Subsequently, 1.3 mL of sodium hydroxide solution (1.0 N) was added to each flask, and the mixtures were diluted to volume with ethanol., the solutions were allowed to react for 15 minutes, and the absorbance was measured at $\lambda_{max} = 410$ nm. As show in the (Figure 3.9) the absorbance increased linearly with the mole ratio up to 1:1 (PPH: reagent), after which the curve reached a plateau with no significant increase in absorbance. This behavior clearly indicates that the reaction between phenylephrine hydrochloride and 2,4,6-trichloroaniline occurs in a 1:1 stoichiometric ratio, confirming the formation of a single azo dye product [27].

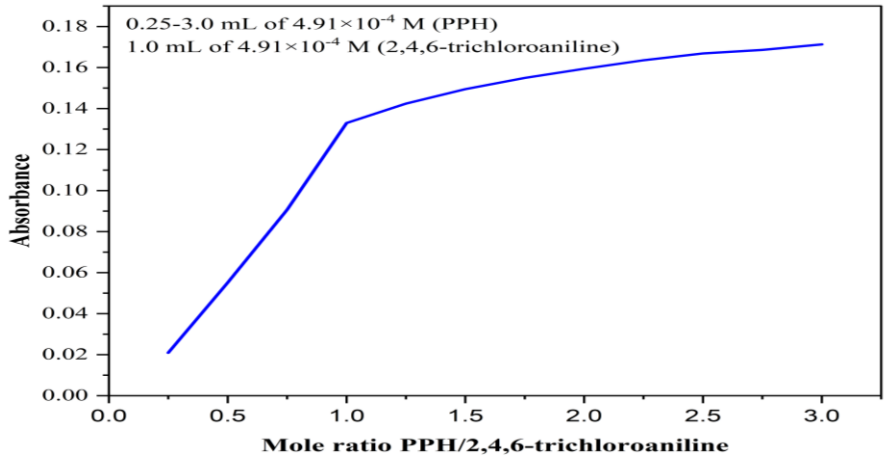


Figure 3.9 Mole ratio plot of phenylephrine hydrochloride (PPH) with 2,4,6-trichloroaniline at $\lambda_{max} = 410$ nm, indicating a 1:1 stoichiometric ratio.

3.6 Application of the Method

The proposed spectrophotometric method was successfully applied for the quantification of phenylephrine hydrochloride (PPH) in its pharmaceutical formulations, yielding satisfactory results. The accuracy and precision of the method were statistically evaluated by comparing the results with those obtained using a reported reference method through t-test and F-test. The statistical calculations were performed at a 95% confidence level, considering n = (number of replicates) for each method. The following equations were used:

$$t = \frac{\bar{d} \times \sqrt{n}}{SD} \qquad (4)$$

$$F = \frac{S_2^2}{S_1^2} \qquad (5)$$

Where $\bar{x}_1$ and $\bar{x}_2$ represent the mean values obtained by the proposed and referenced methods, respectively, S_1^2 and S_2^2 are the corresponding variances, and S_p is the pooled standard deviation. The obtained results indicated that the calculated t and F values were lower than the theoretical values, confirming that there is no significant difference between the proposed and reference methods in terms of accuracy and precision. Table 3.4 present a statistical comparison of the developed method with the official British Pharmacopoeia method. Table 3.5 present a comparison between the proposed method and previously reported spectrophotometric methods for the determination of the drug in pharmaceutical preparations. The proposed method using 2,4,6-trichloroaniline shows a high correlation coefficient and good sensitivity with a suitable linear range, demonstrating comparable or improved analytical performance relative to earlier methods.

Table 3.4. Comparison of the proposed spectrophotometric method with the official reference method (British Pharmacopoeia) for the determination of phenylephrine hydrochloride (PPH) in nasal drop formulations.

Pharmaceutical preparation	µg PPH present/ 10 mL	µg PPH found/10 mL (Proposed Method)	µg PPH found/10 mL (HPLC method)	t-test and F-test
NAZAFRINE nasal drops (%1.0) safa	4.0	3.97	4.12	t-test = 4.00
Nasofen sterile nasal drops (%1.0) pioneer	4.0	3.99	4.02	F-test = 4.00

([a]); average of (n=4) replication.

Table 3.5 Comparison of the proposed spectrophotometric method with previously reported methods for the determination of the drug in pharmaceutical preparations using different reagent.

Reagent	Sample	λ_{max} (nm)	Correlation coefficient (R^2)	Linearity (µg/ml)	Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	Ref.
2-aminobenzothiazole	Nose drop	510	0.9988	10-250	6.620×10^3	(Othman and Abdul Fatah, 2009)
p-nitroaniline	Nasal drops & tussilet	490	0.9981	10-200	1.14×10^4	(Ibraheem, 2009)
Metoclopramide hydrochloride	Nasal drops	470	0.9987	1-32	9.51×10^3	(Al-Abachi and Abed, 2015)
Sulfacetamide sodium	Nasal drops	425	0.9929	2-24	3.442×10^3	(Wasan, 2019)
2,4,6-trichloroaniline	Nasal drops	410	0.9997	0.5-12	1.58×10^4	This study

4. Conclusion

The proposed spectrophotometric method provides a sensitive, accurate, and cost-effective approach for the quantitative determination of phenylephrine hydrochloride (PPH) in pharmaceutical formulations. It allows precise measurement of PPH in both the pure state and nasal drop dosage forms without interference from excipients, and requires neither sample pretreatment nor temperature control. The method is based on a highly stable diazotization-coupling reaction that produces a yellow azo dye with maximum absorbance at 410 nm under optimized conditions, ensuring excellent reproducibility and robustness. It exhibits a linear response over the concentration range of 0.5–12.0 µg/mL, with low detection and quantification limits of 0.1289µg/mL and 0.0425 µg/mL, respectively. The method shows high precision, with a relative standard deviation of 0.16%, and high accuracy, with an overall recovery of 99.86%. Stability studies confirmed that the color remains stable for up to 60 minutes, providing flexibility in analysis. Comparative statistical evaluation against the official HPLC method revealed no significant differences, demonstrating the reliability and suitability of this method for routine quality control. Overall, this simple, efficient, and economical procedure represents a practical alternative for the routine determination of phenylephrine hydrochloride in pharmaceutical samples.

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Author Contributions

Skala Sirwan Saleem conceived the study, performed all experimental work, analyzed the data, and wrote the manuscript. All figures, tables, and supplementary materials were prepared by them. Nabil Adil Fakhre supervised the study, provided critical feedback throughout the research process, edited the manuscript, and contributed to the discussion of the findings. All authors have read and approved the final manuscript.

Data Availability

No new datasets were generated or analyzed in this study.

Declarations

Competing Interests

The authors have no conflicts of interest.

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