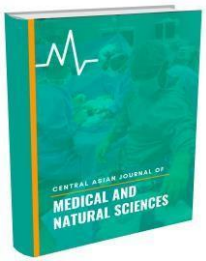

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Article

Spectrophotometric Determination of Phenylephrine Hydrochloride in Pharmaceutical Formulations Using an Oxidative Coupling Reaction

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Abstract: This study presents a simple and accurate analytical method for the quantitative determination of selected decongestants and cough suppressants, specifically phenylephrine hydrochloride, in both pure form and pharmaceutical formulations. The method is based on an oxidative coupling reaction with p-aminophenol using potassium iodate as an oxidizing agent in a hydrochloric acid medium. Key experimental parameters including reagent concentrations, acid strength, temperature, reaction time, and stability were thoroughly investigated and optimized. The developed method demonstrated excellent linearity in the range of 5–50 μg/mL at a wavelength of 548 nm, with high precision, accuracy, and sensitivity evidenced by a low limit of detection (LOD) of 0.1207 μg/mL and a limit of quantitation (LOQ) of 0.3659 μg/mL . Successful application to a commercial syrup formulation yielded high recovery percentages, confirming the method's accuracy and revealing no significant interference from common excipients. Consequently, this proposed method is straightforward, cost-effective, and highly suitable for routine quality control analysis in pharmaceutical laboratories.

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1. Introduction

The chemical analysis of pharmaceutical mixtures presents a significant challenge, particularly when relying on direct spectroscopic techniques such as ultraviolet (UV) spectroscopy, due to substantial spectral overlap among different compounds. To address this issue, a range of advanced methods employing digital and mathematical processing of spectral data have been developed. The success of these techniques largely depends on the extent of spectral interference and the number of components present in the sample [1-5].

Meanwhile, the pharmaceutical industry has witnessed remarkable advancements in designing combination dosage forms that contain multiple active ingredients. This complexity necessitates the development of precise and sophisticated analytical methodologies capable of accurately separating and quantifying each component [6-8]. Among these modern techniques holds a pivotal role as a powerful and reliable tool for the separation and analysis of constituents within complex formulations. This study aims to develop integrated analytical approaches, including ultraviolet (UV) spectroscopy to effectively separate and quantify a selected group of decongestants and cough

suppressants in their pure forms as well as within multi-component pharmaceutical preparations [8-11].

The next stage is detection, which is most commonly performed using ultraviolet (UV) light. Most chemical compounds absorb UV light at specific wavelengths. According to the Beer-Lambert Law, there is a direct relationship between the amount of absorbed light and the concentration of the compound. Initially [12], reference solutions with known concentrations of the target molecule—known as standards—are prepared and passed through the UV detector to record their responses. These values are then used to construct a calibration curve that illustrates the relationship between the molecule's concentration and the UV absorbance response. When analyzing an actual sample, this curve allows the conversion of the detected signal into an accurate concentration of the compound being studied[13],[14], By integrating the processes of separation and detection ,UV technology enables the passage of individual molecules one by one through the separation column, followed by the measurement of each molecule's concentration based on its UV absorbance at the column's outlet. This methodology represents one of the most widely used and precise systems in the bioanalysis of pharmaceutical substances [15-18].

2. Materials and Methods

All spectrophotometric measurements were performed using a UV–Visible spectrophotometer equipped with 1 cm quartz cells. A high-precision analytical balance (0.1 mg) was used for weighing, and all volumetric glassware was of analytical grade , Phenylephrine hydrochloride(PHE) of analytical purity was used as the analytic. Hydrochloric acid (HCl, 1.0 M) was prepared by appropriate dilution and served as the acidic medium. p-Aminophenol(4-AP) of analytical grade was employed as the chromogenic reagent, while potassium iodate was used as the oxidizing agent. All reagents and solutions were prepared using freshly distilled water. A standard stock solution of phenylephrine hydrochloride was prepared at a concentration of 250 ppm by accurately weighing the required amount, dissolving it in distilled water, and completing the volume in a volumetric flask. The p-aminophenol reagent was prepared at a molar concentration of (0.001) M in an acidic medium using (0.1M) HCl. Potassium iodate was prepared at the appropriate molar concentration (0.001 M) in distilled water. Hydrochloric acid at 1.0 M was used for pH adjustment and to create the optimal acidic conditions for the reaction. In the general procedure, the specified volumes of phenylephrine solution, p-aminophenol reagent, hydrochloric acid, and potassium iodate were added into a 25 ml volumetric flask in the optimized order. The volume was completed with distilled water. The reaction mixture was allowed to stand for the optimal reaction time under the determined conditions, and the absorbance was measured at the maximum wavelength (λmax = 548 nm) against a reagent blank prepared in the same manner without the analytic.

General Procedure

In the recommended procedure, an accurately measured volume of phenylephrine hydrochloride solution was transferred into a 25 mL volumetric flask, followed by the addition of a fixed volume of p-aminophenol reagent and hydrochloric acid to provide the acidic medium. Subsequently, a measured volume of potassium iodate solution was introduced as the oxidizing agent to initiate the oxidative coupling reaction. The mixture was allowed to stand under the optimized conditions of time and temperature to ensure complete color development. Finally, the solution was diluted to the mark with distilled water, and the absorbance of the resulting orange-colored product was measured at λmax = 548 nm against a reagent blank prepared in the same manner but without the drug. The absorption spectrum of the formed product is shown in Figure 1.

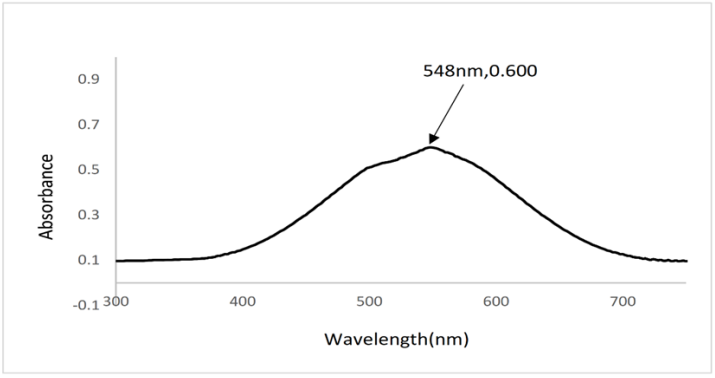


Figure 1. The absorption spectrum of the color product.

3. Results and Discussion

Study of Optimal Reaction Conditions

The effect of diverse variables on the absorbance intensity on the phenylephrine hydrochloride and the optimum conditions have been selected as follows:

1. Selection the best acid

   The coupling reaction between the 4-AP reagent and the PHE solution occurs in an acidic medium, so the effect of various acids was investigated to determine which of them produced the higher absorbance. From the results in Table 1, it is clear that hydrochloric acid gives the highest absorbance. Therefore, it was adopted in subsequent experiments.

Table 1. Selection the best acid.

Type of acid	Wavelength	Absorbance
HCL	548	0.598
H_2SO_4	430	0.282
HNO_3	660	0.249

2. Effect of acid Volume

   This study was conducted to select the best amount of acid solution by adding different volumes (0.5-3.0 mL) of HCl, it was found that 1.5 mL of HCl gives the highest absorbance at pH=1.38 so, the volume was adopted in the later experiments. The results are shown in Table 2.

Table 2. Experiment to select the best volume for HCl acid.

Acid volume(ml)	Absorbance
0.5	0.550
1	0.582
1.5	**0.599**
2	0.469
2	0.430
3	0.385

3. Selection the best reagent

   Among tested reagents, p-aminophenol produced the highest absorbance (0.595), showing strong compatibility with phenylephrine hydrochloride for

oxidative coupling. Its electron-donating properties facilitate stable, highly absorbing product formation.

Table 3. Effect of the volume of reagent.

Reagent	Wavelength	Absorbance
P-bromo anilin	NO.R	NO.R
P-amino anilin	460	0.041
P-amino phenol	**548**	**0.595**
O-aminophenol	390	0.043

4. Effect of Reagent Volume

A study was carried out to establish the optimum amount of reagent solution (4-AP), which gives the maximum absorption of the colored product by adding different volumes (0.5-3.0 mL) of 4-AP reagent (1×10-3M) to the volumetric flasks containing 3.0 mL of PHE solution, 1.0 ml of the potassium iodate (1×10-3M), and 1.5 mL of hydrochloric acid, the volume was completed to 25 mL with distilled water. Table 4 shows that the optimal reagent volume is 1.5 mL, which produced the highest absorbance of the formed product.

Table 4. Effect of Reagent Volume.

Amount(ml) of reagent $1x10^{-3}M$	Absorbance
0.5	0.375
1	0.533
1.5	**0.602**
2	0.550
2	0.415
3	0.395

5. Selection the best Oxidizing Agent

Potassium iodate yielded the highest absorbance (0.600) at 548 nm, outperforming other oxidants. Its strong oxidizing capability in acidic conditions promotes complete and efficient chromophore formation.

Table 5. Finding the best oxidizing agent.

Type of acid	Wavelength	Absorbance
Potassium Iodate	548	0.600
Potassium per Iodate	640	0.003
Potassium per sulphat	550	0.023

6. Effect of Oxidizing Agent Volume

This study was conducted to select the best amount of oxidizing agent (1×10-3M) by adding different volumes (0.5-3.0 mL) of oxidizing agent to volumetric flasks containing 3.0 mL of PHE solution and 1.5 mL of reagent solution in the presence of hydrochloric acid, the volume was completed to 25 mL with distilled water, and measured at wavelength 548 nm. The results shown in Table 6, that the

best volume of oxidizing agent is 1.0 mL. So, it was used in subsequent experiments.

Table 6. Effect of Oxidizing Agent Volume.

Amount(ml) of Oxidant Agent 1x10-^{3}M	Absorbance
0.5	0.583
1	**0.603**
1.5	0.563
2	0.545
2	0.519
3	0.483

7. Effect of Temperature

The effect of temperatures (20–60°C) on the oxidative coupling reaction has been studied using the optimal conditions obtained from previous experiments. We note in this study that the absorbance reached its maximum at a temperature of 30°C. Therefore, the temperature was used in subsequent experiments, the results are shown in Table 7.

Table 7. Experiment on the effect of temperature on absorption.

Temperature C^{o}	Absorbance
20	510
30	**0.600**
35	0.573
40	0.412
45	0.323
50	0.258
55	0.166
60	0.124

8. Effect of Time

The color intensity reached the maximum, after drug was reacted with 4-AP and potassium iodate for 2.0 min in an acidic medium. Therefore, 2.0 min was sufficient for the coupling reaction to be completed, so it was adopted in the subsequent experiments, the results are shown in Table 8.

Table 8. Effect of Time on the Stability.

Time(min)	Absorbance
1	0560
2	**0.610**
5	0.585
10	0.579
15	0.575

9. Effect of stability time on the colored product

The stability of the reaction product was studied by taking volume 3.0 mL of PHE solution and adding 1.0 mL of oxidizing agent, 1.5 mL of 4-AP reagent, and 1.5 mL of HCl in a volumetric flask of 25 mL, then complete the volume to the mark with distilled water. The absorption was measured at the wavelength of 548 nm, where the results showed that the absorption value of the colored product remains stable for 50 minutes. The results are shown in Table 9.

Table 9. Effect of Time on the Stability.

Time (min)	Absorbance
2	0.599
5	0.598
10	0.598
15	0.597
20	0.597
25	0.596
30	0.595
35	0.595
40	0.596
45	0.595
50	**0.595**
55	0.513
60	0.501

10. Addition Sequence

The effect of various orders to choose the utmost sequence in addition to the reactants, it was found from the results that the order No. 5, (D + A + O + R) is the best order to form a colored product with maximum absorbance. Therefore, was chosen in the later experiments, as shown in Table 10.

Table 10. Effect of Addition Sequence.

Addition Sequence	Absorbance
D+O+R+A	0.482
O+ R+D+A	0.495
R+D+O +A	0.554
A+ D+O+ R	0.566
D+A+O+R	**0.603**

(D) Drug solution, (R) Reagent solution, (O) Oxidizing agent solution, (B) Base solution

11. The final absorption spectrum

The final absorption spectrum was measured by using 3.0 mL of PHE, 1.0 mL of potassium iodate (1×10-3 M), and 1.5 mL of 4-AP reagent solution(1×10-3M) in the presence of hydrochloric acid solution (0.1 M) at a temperature of 30°C and the solution was left for 2 min to complete the reaction, then complete the volume to 25 ml in a volumetric flask. The absorption was measured against the blank solution, it was found that it gives the highest absorption at the wavelength of 548 nm, while its blank solution gives a few absorptions at the same wavelength, the results are shown in Figure 2.

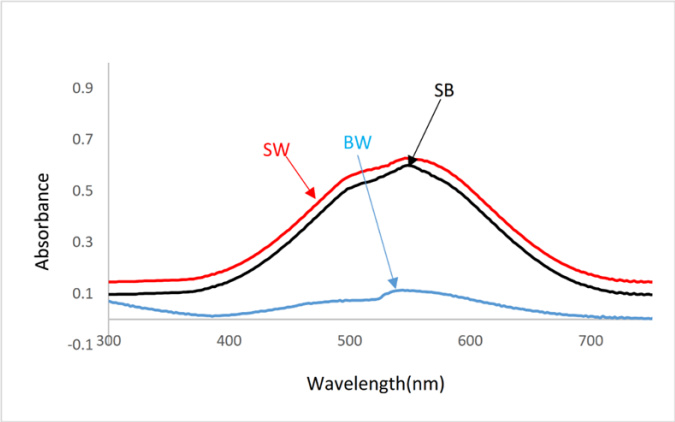


Figure 2. The final absorption spectrum for the determination of PPE , compared to distilled water (SW), PPE compared to blank solution (SB), and Blank solution compared to distilled water (BW).

Table 11. The optimum conditions for the determination of PPE.

Parameters	Value
λ max	548 nm
Amount of PPE	3mL
P- aminophenol (1x10^{-3} M)	1.5mL
potassium iodate (1x10^{-3} M)	1.0 mL
Amount mL of 0.1 HCl	1.5mL
Temperature	30 C°
Solvent	Water
Reaction time	2 min
Reaction order	D+A+O+R

(PPE) Drug solution, (R) Reagent solution, (O) Oxidizing solution, (A) acid solution

12. Working Method and Calibration Graph and Curve

After selecting the optimized experimental conditions shown in Table 11, PHE solution (5-50 µg/mL) was transferred to a series of volumetric flasks (25 ml), followed by 1.0 mL of oxidizing agent solution, 1.5 mL of hydrochloric acid, and 1.5 mL of 4-AP reagent, the solutions have been left for 2.0 min to complete the reaction, and then the volumes were completed to the mark with distilled water. The absorbance of the solutions was measured against the blank solution at a wavelength of 548 nm. Figure 3, and Figure 4, clear that the calibration curve is linear over the concentration range (5- 50 µg/ml), while higher concentration shows a negative deviation from Beer's law. The molar absorption coefficient was 3.828 ×103 L.mole-1.cm-1, and the Sandel sensitivity value was 0.0531 µg/cm2.

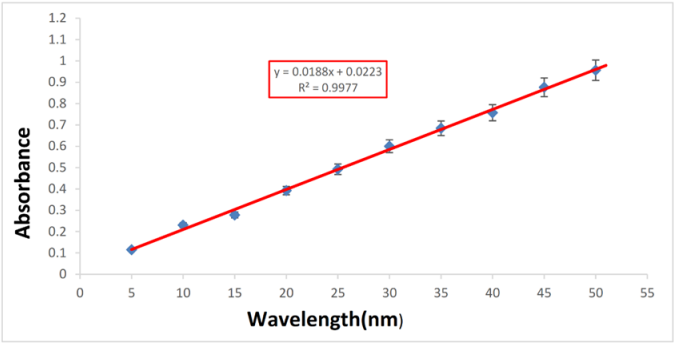


Figure 3. The calibration graph for the PPE drug.

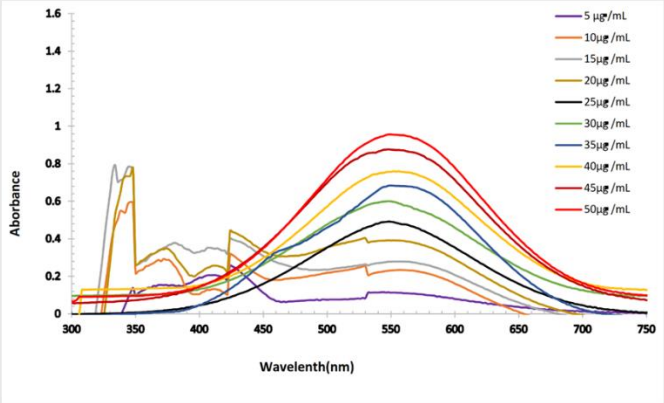


Figure 4. Calibration curve of PPE.

13. The Nature of the Formed Product

To know the nature of the red-orange color product (stoichiometry of drug with the reagent), Job's method and the molar ratio method were applied. In both methods, the concentration of the PHE solution and the 4-AP reagent solution was equal to 1.23×10-3M.

14. Continuous Variation Method (Job's Method)

In a series of volumetric flasks (25 ml), different volumes of the PHE drug solution ranging from (0.5-4.5 mL) and different volumes (4.5-0.5 mL) of 4-AP reagent solution were mixed, 1.0 mL of potassium iodate and 1.5 mL of hydrochloric solution was added. The volumes were completed to the mark with distilled water, and then the absorbance was measured at 548 nm against the blank solution. The results in Figure 5, showed that the ratio is 1:1.

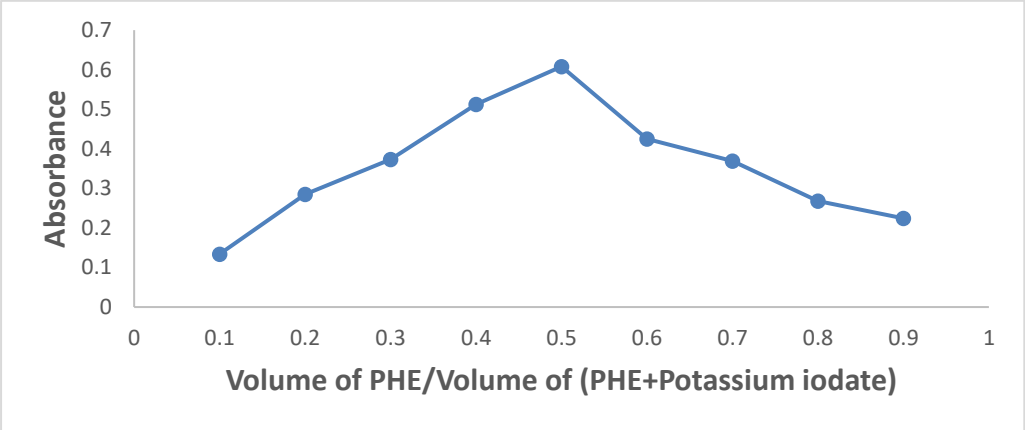


Figure 5. Method of continuous.

15. Molar Ratio Method

3.0 mL of the standard drug solution in a series of volumetric flasks 25 ml was transferred and different volume (0.5-5.0) mL of 4-AP reagent solution in the same concentration of PHE, 1.0 mL of potassium iodate (1×10-3M), and 1.5 ml of hydrochloric acid solution (0.1 M) were added. The volumes were completed to the mark with distilled water, and the absorbance was measured at 548 nm against the blank reagent. The results in Figure 6, which is in agreement with the Job's method results.

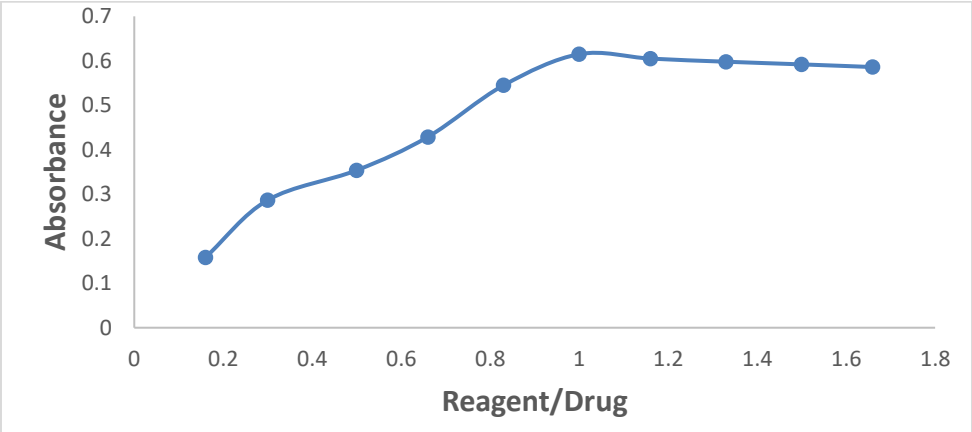
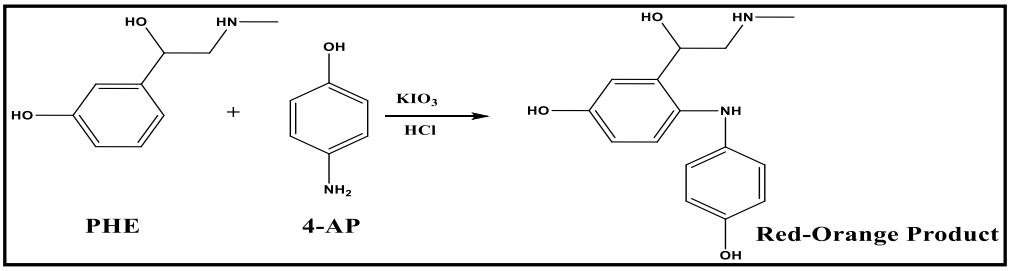


Figure 6. Mole ratio method of PPE.

The proposed equation for the dye formed by the reaction of PHE with the 4-AP reagent was shown in scheme 1.



Scheme 1. The mechanism of the proposed method .

16. Precision and Accuracy

The examination of the precision and accuracy of the approach involved the assessment of the recovery percentage, as well as the relative standard deviation (RSD%) and the relative error (RE%) across three distinct concentrations (10, 30, and 35) µg/mL, with the absorbance being assessed six times at a wavelength of 548 nm for each concentration. Subsequently, an average was computed. The results presented in Table 12 indicated that the methodology employed to determine PHE exhibited satisfactory precision and accuracy.

Table 12. The results of precision and accuracy.

Amount of BHH µg/mL		*RE%	*Recovery%	*RSD%
Taken	Measured			
10	10.62	0.55	100.55	1.21
30	30.05	0.83	100.83	0. 972
35	35.015	1.25	101.25	1.30

*Average of six determinations

17. The detection limit (LOD) and quantification limit (LOQ)

The limits of detection and limits of quantitation were determined by measuring the absorption of the blank solution six times at a wavelength of 548 nm under optimal conditions1-3. The results are shown in Table 13.

$$LOD=\frac{3.3\times SD}{b} \quad \text{.....(1)}$$

$$LOQ=\frac{10\times SD}{b} \quad \text{.....(2)}$$

Table 13. LOD and LOQ values.

Absorbance of blank *	Standard deviation (SD)	Slope (b)	LOD	LOQ
0.11	0.000688	0.0188	0.1207µg/mL	0.3659 µg/mL

*Six determinations

18. Phenylephrine Hydrochloride Syrup Formulation Solution (250 µg/mL)

The analytical study targeted the pharmaceutical formulation (RivoRaz® syrup) containing Phenylephrine HCl, a nasal decongestant. The sample was purchased from a pharmacy and manufactured by Al-Razi, Aleppo, Syria in Syria. The formulation contained Phenylephrine HCl with a concentration of 5 mg/5 mL, which is equivalent to 1 mg/mL (0.001 g/mL). To prepare a working solution with a concentration of 25 ppm, a volume of 25 mL of the stock solution was measured and diluted to 100 mL with distilled water to obtain a final concentration of 250 ppm.

19. Applications

The method was applied to a pharmaceutical formulation containing Phenylephrine HCl: RivoRaz® syrup (Al-Razi, Aleppo, Syria), labeled 5 mg/5 mL.

20. Direct method

Three different concentrations (10, 25, and 45 µg/mL) were prepared from the 250 µg/mL syrup solution and treated as described in the calibration procedure. Absorbance was measured at the selected wavelength, and recovery, relative error (RE%), and relative standard deviation (RSD%) were calculated.

Table 14. The results of the direct method for the determination of PHE in pharmaceutical preparation.

Concentration µg/mL Taken	Concentration µg/mL Measured	RE%*	Recovery*	RSD%*
10	9.95	-0.5	99.5	0.860
25	25.11	0.44	100.44	0.760
45	44.77	-0.51	99.48	0.485

*Average of Six determinations

21. The Standard Addition Method

To prove that the developed method was free from interferences, the method of standard additions was applied to determining PHE in its pharmaceutical preparation. The addition of constant volumes (2.5 mL) which, is equivalent to (25 µg/mL) of pharmaceutical solution in two series of six volumetric flasks of 25 ml, then adding increasing (1 to 5.0 mL) of PHE solution. The solutions were treated in the same way as at the calibration curve, and then the absorption was measured against the blank solution at the wavelength 548 nm. The results are shown in Table 15, and Figure 7.

Table 15. The results of the standard addition method for the determination of PHE in pharmaceutical preparation.

Concentration µg/mL Taken	Concentration µg/mL Measured	RE%	Recovery %
25	24.83	-0.64	99.36

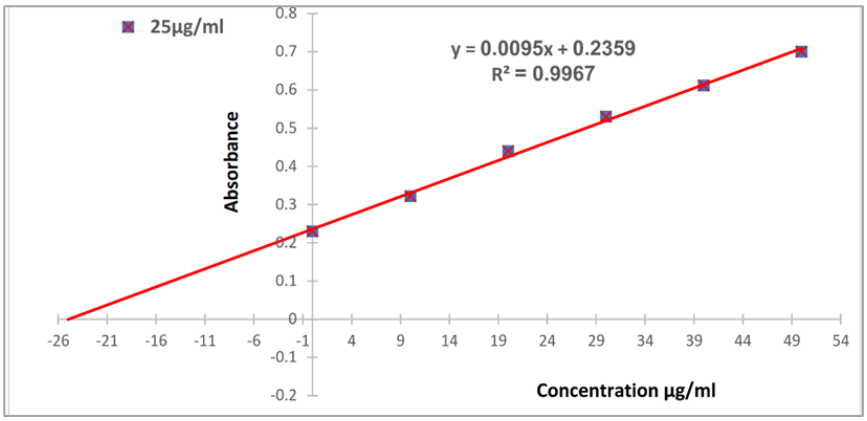


Figure 7. The standard addition method.

4. Conclusion

The developed spectrophotometric method using oxidative coupling with p-aminophenol and potassium iodate provides a simple, accurate, and reliable procedure for the determination of phenylephrine hydrochloride in bulk and dosage forms. The optimized conditions ensured high sensitivity, good stability, and excellent reproducibility. Application to commercial formulations confirmed its accuracy and freedom from matrix interference. Due to its cost-effectiveness and straightforward procedure, the method is recommended for routine pharmaceutical quality control and can be adapted for the analysis of similar active pharmaceutical ingredients in complex formulations.

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