International Journal of Chemical and Pharmaceutical Research Updates

Journal homepage: https://orionjournals.com/ijcpru/

(Research Article)

Spectral identification of epinephrine hydrochloride in pharmaceutical preparations using the oxidative coupling reaction

Ahmed A. Qanbar *

Collage of Education – Tuz Khurmatu, Tikrit University, Salah Al-Din, Iraq.

International Journal of Chemical and Pharmaceutical Research Updates, 2026, 07(01), 072–082

Publication history: Received on 23 June 2026; revised on 29 July 2026; accepted on 31 July 2026

Article DOI: https://doi.org/10.53430/ijcpru.2026.7.1.0046

Abstract

In this research, a precise and straightforward analysis method has been proposed to quantitatively analyze selected decongestants and antitussives such as epinephrine hydrochloride in pure and medicinal forms. In this method, the oxidative coupling reaction of p-aminophenol is achieved with the use of potassium iodate in the presence of hydrochloric acid solution. Various experimental conditions such as concentration of reagents, acidity, temperature, time, and stability have been explored and optimized. The technique showed good linear calibration graph in the range of 5–50 µg/mL at λ= 550 nm with high accuracy, precision and sensitivity with LOD of 0.1301 µg/mL and LOQ of 0.3680 µg/mL. Application to commercial syrup preparation showed good percentage recoveries, indicating that the accuracy of the technique and showing no interference due to commonly used excipients. Thus, the developed method is very easy, economical and appropriate for routine quality control studies in pharmaceutical laboratories.

Keywords: PPE; Pharmaceutical; Hydrochloride; Epinephrine; Reagents; UV Absorbance; Spectrophotometric; Drug

1 Introduction

The determination of composition in pharmaceutical blends is not an easy task, especially using direct spectroscopy, which includes the ultraviolet spectroscopy method, because of the interference of the spectra of different substances. For that reason, various methods of digital and mathematical spectral data processing have been created. The performance of such approaches is highly dependent on the amount of the interference and the number of compounds [1-5]. However, there have been incredible improvements in the pharmaceutical industry in the creation of combination dosage forms that have many active components [6-8]. This calls for advanced techniques of analysis for accurate separation and quantification of the individual components [6-8]. Advanced techniques of analysis include one which is crucial for the separation and analysis of the components in complicated mixtures [6-8]. The purpose of this study is the development of advanced techniques of analysis that will be used in the separation and analysis of decongestants and cough suppressants in their pure state and also when included in medications [8-11]. The next step is that of detection and this is normally accomplished using UV light. Almost all the chemicals absorb light in the UV region in different ranges. There is an existing relationship according to Beer Lambert Law that states that there is a direct relationship between the amount of light absorbed and the concentration of the substance. Initially [12], the solutions whose concentrations of the substance are known are referred to as the standards. This is done in order to make the calibration curve showing how the concentration of the substance and its response are interrelated. In case of sample analysis, the curve can be used for conversion of the detected response into the concentration of the substance under study [13][14]. Using both the techniques of separation and detection, the technique of UV allows each molecule to pass individually through the separation column, and further measuring their concentrations using UV absorbance at the outlet of the column. The approach is considered one of the most popular and precise approaches in the bio-analysis of drug substances [15-18].

* Corresponding author: Ahmed A. Qanbar. ahmed.abd.tuz@tu.edu.iq

2 Material and methods

Spectrophotometric analysis was carried out using a UV visible spectrophotometer with 1 cm quartz cells. Highly accurate balance with precision to 0.1 mg was used for weighing of the chemicals. All glasswares used for the measurements were of analytical class. The analyte employed in the present experiment was epinephrine hydrochloride (PHE), an analytically pure substance. Acid medium was hydrochloric acid (HCL), diluted to a concentration of 1.0M. Chromogenic reagent employed in the experiment was p-amino phenol (4-AP), an analytically pure substance. Oxidizing agent was potassium iodate. Stock solution of epinephrine hydrochloride was prepared by weighing appropriate amount of the substance and dissolving it in distilled water and volume was made up to mark using volumetric flask of 200 ppm concentration. Preparation of p-aminophenol reagent was prepared using molarity of (0.001) M in acidic medium of (0.1M) HCl. Preparation of Potassium Iodate was prepared using molarity of (0.001M). 1.0M HCl was used for pH adjustments and preparation of acidic medium. As per the process, suitable amount of Epinephrine, p-aminophenol reagent, HCL and Potassium Iodate were taken in 20 ml volumetric flask in proper sequence . The blank solution was prepared in accordance with the process described above, except that no analyte was added into the solution. Distilled water was used to bring the solution to the mark. The reaction solution was allowed to react undisturbed, and its absorbance was determined at λ max = 550nm.

3 General Procedure

The procedure suggested here implied the addition of a proper quantity of the epinephrine hydrochloride solution to the 20 mL volumetric flask, after which an adequate quantity of the p-aminophenol reagent and hydrochloric acid should be used to guarantee an acidic nature of the solution. After that, the potassium iodate solution of a definite concentration should be added to guarantee oxidation and, consequently, the initiation of the reaction. The solution was kept under certain conditions for some time to assure complete coloring. In the next step, The dilution was made until the mark was reached with distilled water, and the absorbance of the orange-coloured compound was measured at λmax = 550 nm by using the blank solution, which was prepared in a similar way as the solution mentioned above, but no drugs were added to it.

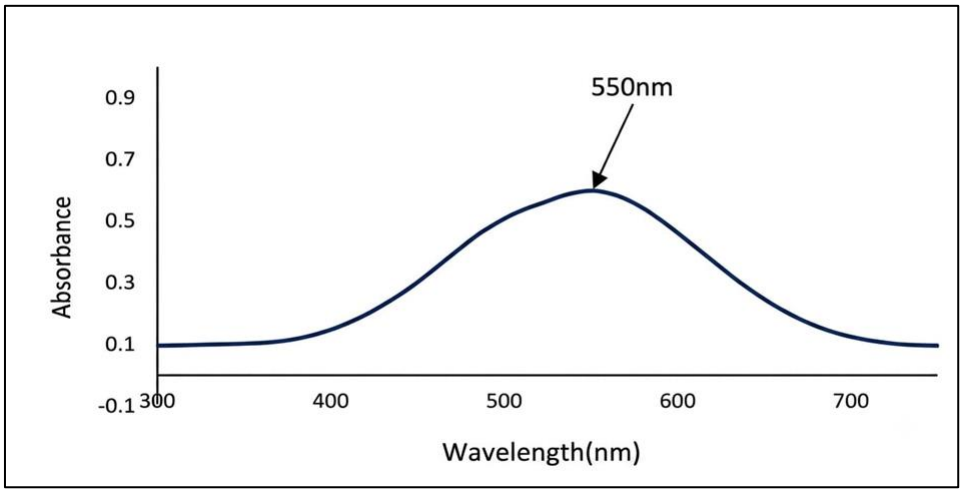

Figure 1 Absorption spectrum of the prepared substance

4 Results and discussion

4.1 A study of the conditions and factors influencing interaction

4.1.1. The best acid

As the coupling process occurs between the 4-AP reagent and PPE in acidic medium, the influence of different acids has been investigated in order to determine the acid providing maximum absorption. It can be observed from Table (1) that maximum absorption is offered by HCl.

Table 1 Selection the best acid

acid	Wavelength	Absorbance
HCL	550	0.590
H_2SO_4	435	0.288
HNO_3	655	0.255

4.1.2. Effect of acid Volume

This research study is done to choose the optimal quantity of the acid solution through using various quantities (1-3.5 mL) of HCl. It was observed that 2 mL of HCl results in maximum absorbance at pH = 1.40, and thus this quantity was used in further experiments.

Table 2 The best volume for HCl acid

Acid size (ml)	Absorbance
1	0.555
1.5	0.587
2	**0.598**
2.5	0.470
3	0.435
3.5	0.388

4.1.3. The best reagent

All the reagents used showed their maximum absorbance value of 0.598 when p-aminophenol was used. It showed maximum absorbance because of its property to donate electrons easily to form stable products.

Table 3 Effect of reagent volume

Reagent	Wavelength	Absorbance
P-bromo aniline	NO.R	NO.R
P-amino aniline	466	0.041
P-amino phenole	**550**	**0.598**
O-aminophenole	395	0.050

4.1.4. Effect of volume reagent

In order to find out the ideal volume of the reagent (4-AP) in which maximum absorbance will occur due to the formation of the colored compound, various volumes of the reagent (4-AP, 1×10-3M) were taken together with 3.5 mL of the PHE solution, 1.0 ml of potassium iodate (1×10-3M) and 1.5 ml of HCl, while keeping the total volume constant at 25 mL with distilled water. As observed from table (4), the ideal volume of the reagent should be 2 mL.

Table 4 Effect Volume Reagent

Reagent amount (ml) of $1x10^{-3}M$	Absorbance
1	0.380
1.5	0.540
2	**0.6010**

2.5	0.555
3	0.420
3.5	0.398

4.1.5. The best Oxidizing Agent

The potassium iodate had the highest absorbance (0.608) at 550 nm compared to other oxidizing agents. The high oxidizing power of the potassium iodate under acidic conditions facilitates efficient formation of the chromophore.

Table 5 Finding the best oxidizing agent

Type of acid	Wavelength	Absorbance
Potasium Iodate	550	0.608
Potasium per Iodate	645	0.005
Potasium per sulphatic	555	0.028

4.1.6. Effect of Oxidizing Volume Agent

The experiment was carried out for the determination of the optimal value of the oxidizing agent (1x10-3 M) through addition of different amounts of the oxidizing agent (1-3.5 mL) in the volumetric flask which contains 3.5 mL of PHE solution and 2 mL of the reagent solution with the help of hydrochloric acid in a volume of 25 mL of distilled water, and the measurement was done at a wavelength of 550 nm. The obtained results (Table 6) indicate that the optimal amount of the oxidizing agent is 1.0 mL.

Table 6 Effect of Oxidizing Agent Volume

Oxidant Agent Amount (ml) 1x10-^{3}M	Absorbance
1	0.585
1.5	0.608
2	0.567
2.5	0.549
3	0.522
3.5	0.486

4.1.7. Effect of Temperature

The influence of different temperatures (from 25 to 65 degrees centigrade) on the oxidative coupling process was studied under optimal experimental conditions identified through earlier studies. In this experiment, it is observed that the maximum absorbance occurs at the temperature of 35 degrees centigrade. Hence, the temperature used in the following experiments is shown in Table (7).

Table 7 Effect of temperature on absorption

Temperature C^{o}	Absorbance
25	510
35	**0.607**
40	0.573
45	0.412
50	0.323

55	0.208
60	0.166
65	0.129

4.1.8. Effect of Time

Maximum intensity of the dye was obtained by the reaction between the drug and 4-AP along with potassium iodate after 4.0 minutes in acidic medium. It can be seen that 4.0 minutes is sufficient for completion of coupling reaction; therefore, it was selected for use in further experiments, as shown in Table 8.

Table 8 Effect Time on the Stability

Time(min)	**Absorbance**
2	0565
4	0.615
10	0.588
15	0.583
20	0.579

4.1.9. Effect stability time on the colored product

Test of the stability of the color compound is done by using 3.5 ml of PHE solution together with 1.5 ml of the oxidant, 2 ml of 4-AP reagent and 2 ml of HCl in a volumetric flask of 25 ml capacity which is filled with distilled water. Absorption is determined at 550 nm. It was found that the value of absorption of the color compound was stable for 55 minutes.

Table 9 Effect Time on the Stability

Time (min)	**Absorbance**
5	0.599
10	0.598
15	0.598
20	0.597
25	0.597
30	0.596
35	0.595
40	0.595
45	0.596
50	0.595
55	**0.595**
60	0.513
65	0.501

4.1.10. Addition Sequence

As for the effect of different orders in choosing the best order as well as the reagents, it has been observed through as the conclusion that the Order No. 5 (D + A + O + R) is the optimum order to achieve the highest absorbance of the color complex formation. Hence it was used in all further experiments as seen from Table (10).

Table 10 Effect of Addition Sequence

Addition Sequence	Absorbance
Du +Ox +R+A	0.487
Ox+ R +Du +A	0.498
R +Du +Ox +A	0.559
A+ Du +Ox+ R	0.568
Du +A+ Ox +R	0.608

(Du) Drug solution, (R) Reagent solution, (Ox) Oxidizing agent solution, (B) Base solution

4.1.11. Absorption spectrum

The absorption spectrum analysis was carried out using 3.5 mL of PPE, 1.5 mL of potassium iodate (1 × 10-3 M) and 2 mL of 4-AP (1 × 10-3 M). Hydrochloric acid was used to aid in the process of mixing. This took place at 35 °C. The absorbance was recorded after two minutes of mixing the solution at 30 °C. The total volume of the solution used in the experiment is 25 mL. From the results obtained above, it is evident that the maximum absorbance is at 550 nm.

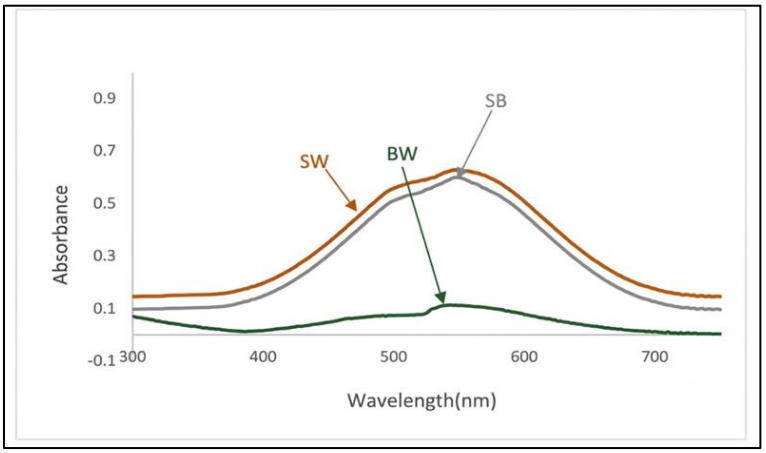

Figure 2 Absorption spectrum at the end of PPE calculation as compared with SW, PPE calculation as compared with SB, and blank solution as compared with SW

Table 11 Optimum conditions determination of PPE

Parameters	***Value***
λ_{max}	*550 nm*
Amount	*3.5 mL*
P- aminopheenol ($1x10^{-3}$ M)	*2 mL*
potasium iodate ($1x10^{-3}$ M)	*1.5 mL*
Amount of HCl (0.2ml)	*2 mL*
Temperature	*35 C°*
Solvent	*Water*
Time of the reaction	*4 min*
Order of the reaction	*Du +A +O+ Re*

(Du) Drug soloution, (Re) Reagent soloution, (O) Oxidiziing solution, (A) acid soloution

4.1.12. Operating procedures, calibration curves and graphs

Upon optimization of the experimental conditions as indicated in Table (11), PPE solution (5-50 µg/mL) was added into several volumetric flasks (20ml), where 1.0mL of oxidant solution, 1.5mL of HCl solution, and 1.5mL of 4-AP solution was added to the solutions. Solutions were left to stand for 2.0 minutes and finally diluted to the mark using distilled water. Absorbances were determined for all the samples using blank solutions through comparative methods. It can be seen from Figures (2) and (3) that the calibration graph has a linear nature in the concentration range (5-50 µg/mL). However, above this range, a departure from Beer's law is noted. Molar Absorptivity has been found to be 3.828 x 103 L.mole-1.cm-1 whereas Sandel sensitivity was 0.0531 µg/cm2.

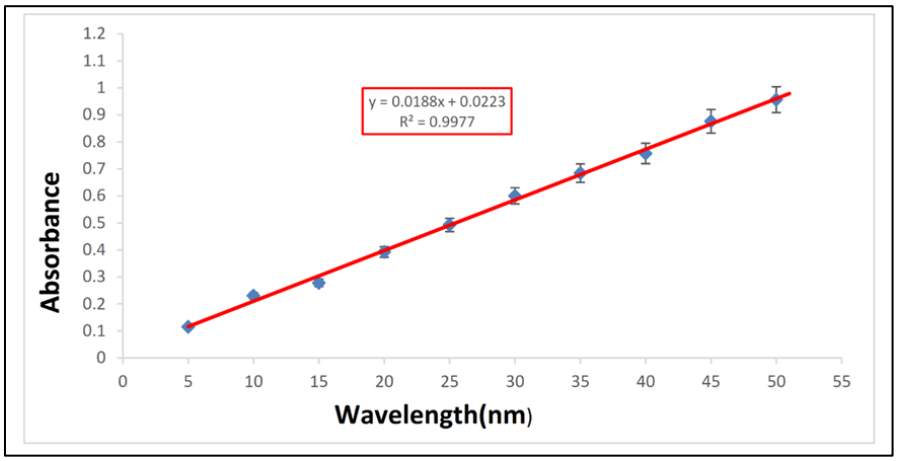

Figure 3 The calibration graph for the PPE drug

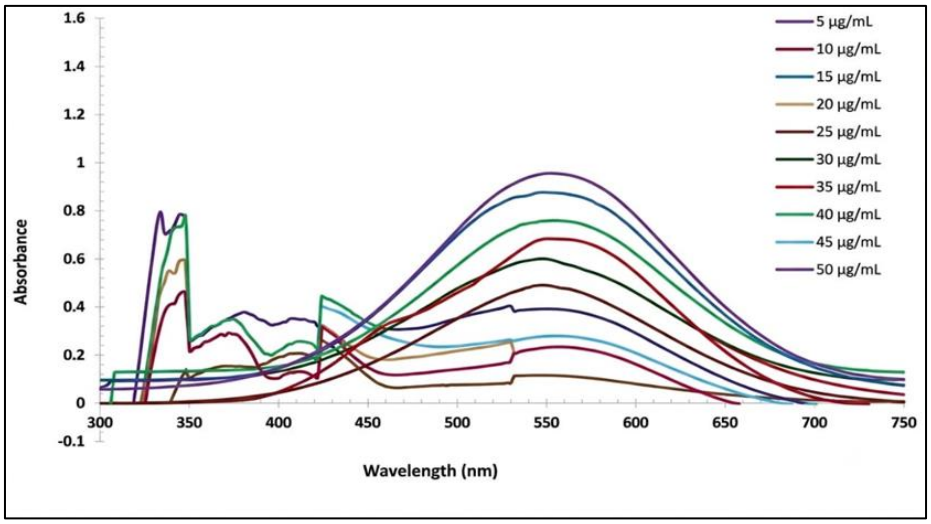

Figure 4 Calibration curve of PPE

4.1.13. Formed Product Nature o

The stoichiometric ratio between the compound and the reagent that produced the red-orange complex was calculated through Job's Method and molar ratio method. In both cases, the concentration of the PHE and 4-AP solution was 1.23×10-3 M.

4.1.14. Method (Job's Method) Continuous Variation

The solutions of PPE drug with varied volumes of 0.5-4.5 mL and solutions of 4-AP reagent with varied volumes of 4.5-0.5 mL were used for mixing in the series of volumetric flask (20 mL). In addition to this, 1.0 mL of potassium iodate

and 1.5 mL of HCl solution were used in the mixture. The mixture was made to volume with distilled water and absorbance was recorded at 550 nm. A 1:1 ratio can be seen in figure 5 below.

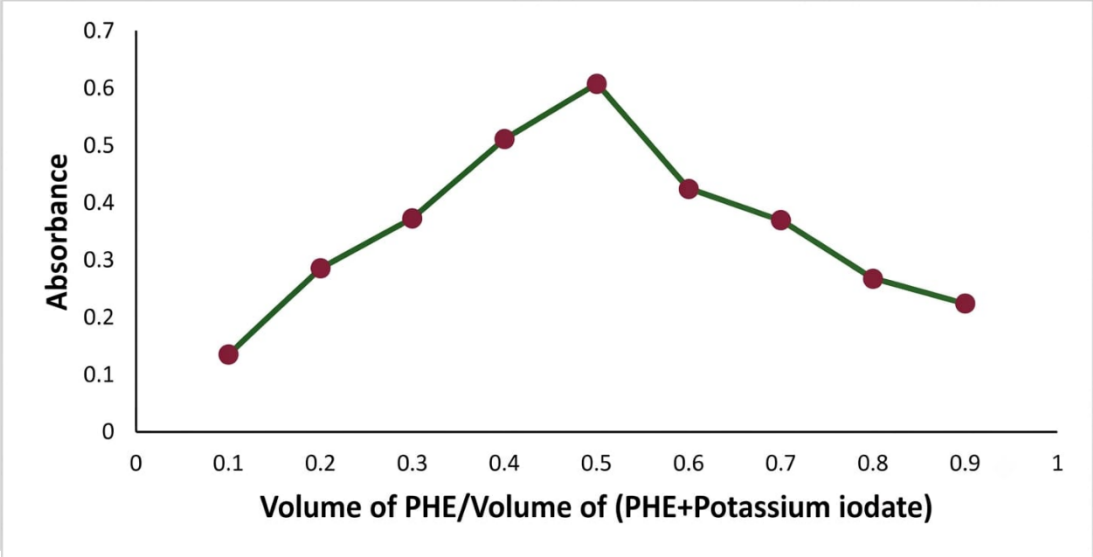

Figure 5 Method of continuous

4.1.15. Molar Ratio Method

Using a series of volumetric flasks of 20 ml, 3.0 ml of the standard drug solution was diluted along with varying volumes (0.5 - 5.0) ml of 4-AP reagent solution of equal concentration of PHE, 1.0 ml of potassium iodate (1×10-3M), and 1.5 ml of hydrochloric acid solution (0.1 M) was taken. The volume was adjusted up to the mark with distilled water and the absorbance was measured at 550nm by taking blank reagent. The results are represented in figure (6) below which is in good agreement with the results obtained by Job's method.

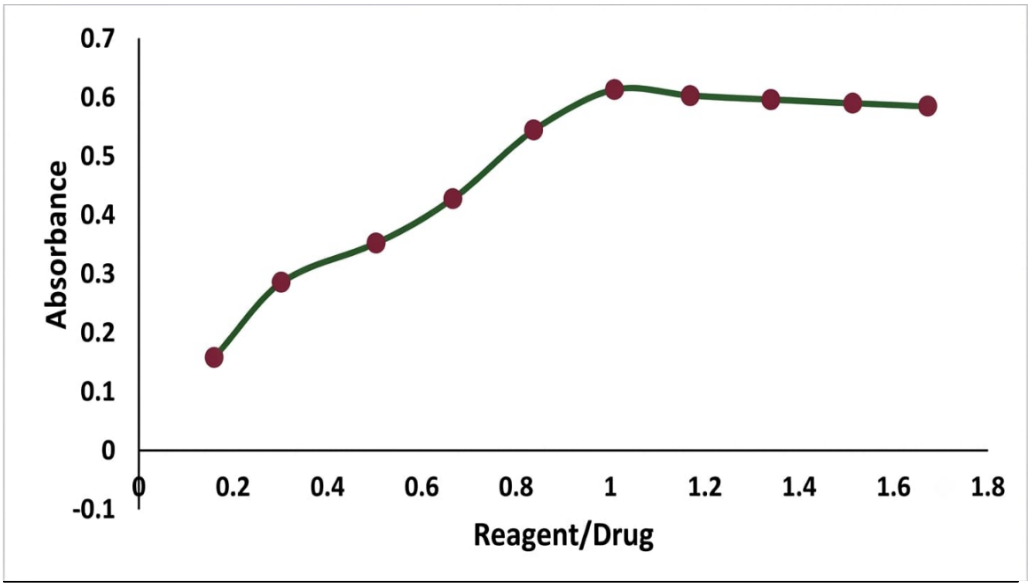

Figure 6 Mole_ ratio method of PPE

4.1.16. Precision and Accuracy

The assessment of precision and accuracy of the method was done through the calculation of recovery %, RSD% and RE% of the three different concentrations (15, 35 and 40) µg/mL, where the absorbance of each concentration was measured six times at wavelength 550 nm. The average value was then calculated. From the results given in Table (12), it can be seen that the method of PHE estimation was precise and accurate.

Table 12 The results of precision and accuracy

Amount of BHH µg/mL		****RE%***	****Recovery%***	****RSD%***
Taken	Measured			
15	12.30	0.75	99.55	*1.32*
35	33.10	0.91	99.99	*0. 992*
40	*38.025*	*1.35*	*100*	*1.41*

*Average of six determinations

4.1.17. 17- Detection Limit (LOD) & Quantification Limit (LOQ)

The detection limits (LOD) and quantification limits (LOQ) have been determined using the six readings of absorbance of the blank solutions at 550 nm wavelength as optimized for the measurements1-3. The obtained results are presented in Table (13).

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

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

Table 13 LOD and LOQ values

Absorbance of blank *	**Standard deviation (SD)**	**Slope (b)**	**LOD**	**LOQ**
0.16	0.000693	0.0194	0.1301µg/mL	0.3680 µg/mL

* Six determinations

4.1.18. Epinephrine Hydrochloride Syrup Formulation Solution (210 µg/mL)

Whereas the subject matter of analytical testing involves RivoRaz® syrup formulation, the active constituent was found to be Epinephrine HCl. The drug was bought from the pharmacy and was made by Al-Razi, Aleppo, Syria. In this connection, the active constituent of the drug is Epinephrine HCl with 5 mg/5 ml concentration or 1.5 mg/ml (0.002 g/ml). In order to make a 25 ppm solution, 25 ml of stock solution was needed for preparation of 125 ml of distilled water.

4.1.19. Applications

The proposed method was used for analyzing Epinephrine HCl in a pharmaceutical formulation: RivoRaz® syrup (Al-Razi, Aleppo, Syria), 5 mg/5 mL.

4.1.20. Direct method

There were three different concentration levels (15 µg/mL, 25 µg/mL, and 50 µg/mL) that were prepared from the 200 µg/mL syrup solution according to the method described above in the calibration process. The absorbance value was measured at the selected wavelength, and the values for recovery, %RE, and %RSD were calculated.

Table 13 Factors determining the direct method for determining the PHE content in a pharmaceutical preparation

Condensed µg/mL Taken	**Condensed µg/mL Measured**	**RE%***	**Recovery***	**RSD%***
15	10.98	-0.55	99.8	0.867
25	23.21	0.48	101.49	0.766
50	45.87	-0.56	99.57	0.489

*Average of Six determinations

4.1.21. Method of Standard Addition

To prove the interference-free nature of the developed method, the standard addition technique was employed for the quantification of PHE in its pharmaceutical form. This was performed by adding equal volumes (2.5 mL) of the pharmaceutical sample, which corresponds to 20 µg/mL in two groups of six volumetric flasks (20 mL each), Subsequently, different amounts (1 to 5.0 mL) of PHE solution were added. The samples were treated as previously mentioned in order to prepare the standard curve and obtain the absorbance values in relation to the blank solution at 550 nm.

Table 14 Determinants of the standard addition method for PHE in pharmaceutical preparations

Condensed µg/mL Taken	Condensed µg/mL Measured	RE%	Reversal %
20	25.83	-0.7	99.58

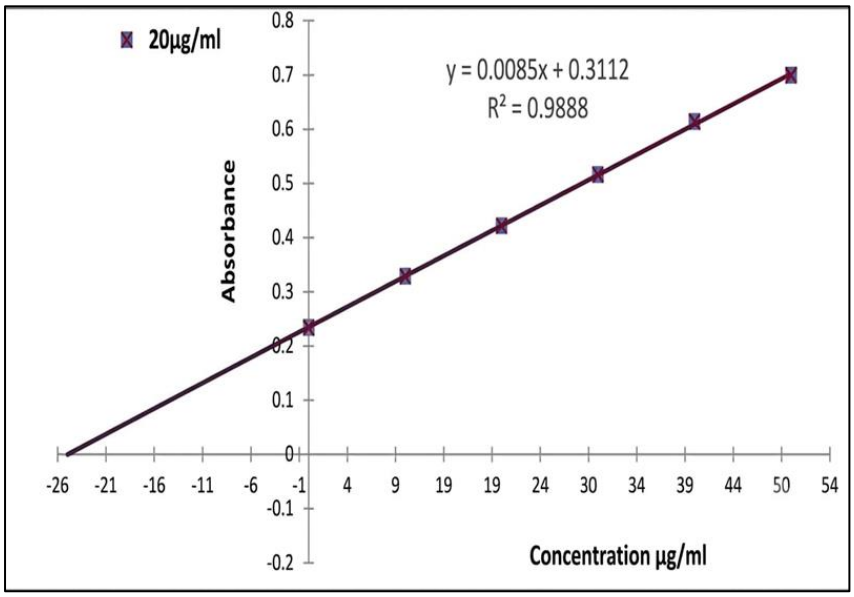

Figure 7 Standard addition methods

5 Conclusion

The spectrophootometric determination method of epinephrine hydrochloride by the oxidation reaction of p-aminophenol and potassium iodate has been proved to be an appropriate, precise and reliable method for the estimation of epinephrine hydrochloride in its pure form and also in medicinal formulations. Under the optimum conditions, maximum sensitivity, stability and reproducibility of the method have been achieved. Precision and accuracy of the method have been evaluated using the drug samples and it has been found to be very precise and not affected by the matrix effect.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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