Determination of Phenylephrine Hydrochloride using Azo-Coupling Reaction and Cloud Point Extraction

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Two simple, sensitive, and accurate spectrophotometric methods were developed for the determination of phenylephrine hydrochloride (PHE·HCl) in its pure form and pharmaceutical dosage forms. In method (A), PHE·HCl reacts with diazotized 3,4,5-trimethoxyaniline (TMA) via a diazo-coupling reaction to form a yellowish-orange azo dye soluble in an aqueous medium, exhibiting a maximum absorbance at 454 nm. Method (B) is based on cloud point extraction, in which Triton X-114 is added to the azo dye solution without altering its color or absorption maximum. The experimental conditions affecting both methods were systematically optimized. Under the optimized conditions, Beer's law was obeyed over concentration ranges of 1–22 μg mL^{-1} and 0.3–12 μg mL^{-1} for methods (A) and (B), respectively. The determination coefficients (R^2) were 0.9993 and 0.9976, while the molar absorptivities were 1.80 × 10^4 m^2 mol^{-1} and 3.73 × 10^4 m^2 mol^{-1} for methods (A) and (B), respectively. Sandell's sensitivities were 1.13 × 10^{-8} kg m^{-2} and 5.4 × 10^{-9} kg m^{-2}. The limits of detection and quantification were 0.08 μg mL^{-1} and 0.29 μg mL^{-1}, respectively. Both methods were successfully applied to the determination of PHE·HCl in pharmaceutical preparations, including drops and tablets, with satisfactory recoveries ranging from 100.34% to 102.48%. In addition, the environmental impact of the proposed methods was evaluated using greenness assessment tools, confirming their eco-friendly analytical profiles.

Keywords: phenylephrine hydrochloride, cloud point extraction, 3,4,5-trimethoxyaniline, spectrophotometry, analytical greenness

Introduction

Phenylephrine hydrochloride ($C_9H_{14}ClNO_2$, PHE·HCl) is chemically known as (1R)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol hydrochloride (Figure 1a) [1]. It is a white crystalline powder belonging to the phenethylamine class and acts as a selective α-adrenergic receptor agonist [2]. Clinically, phenylephrine hydrochloride is used to relieve symptoms without affecting the underlying cause of the condition [3]. It is administered either alone or in combination with other agents, such as tropicamide [4]. The drug is commonly prescribed to increase blood pressure [5], alleviate symptoms of colds and coughs by reducing mucus viscosity and increasing sputum volume, and relieve nasal congestion [6–8].

Owing to its therapeutic importance, numerous analytical methods have been reported for the determination of phenylephrine hydrochloride, including high-performance liquid chromatography (HPLC) [9–11], flow injection analysis [12,13], electrochemical methods [14,15], and conductometric titration [16]. However, many of these techniques require expensive instrumentation, skilled operators, and are not always available in routine analytical laboratories. In contrast, spectrophotometric methods are widely favored because of their simplicity, sensitivity, and cost-effectiveness. Several spectrophotometric procedures for the determination of phenylephrine hydrochloride have been reported, including diazotization–coupling reactions [17–22], oxidative coupling reactions [23], oxidation–reduction reactions [24], and derivative spectrophotometry [25,26]. Moreover, a variety of chromogenic reagents have been employed to enhance the selectivity and sensitivity of these methods [27,28].

In the present study, two sensitive spectrophotometric methods are proposed for the determination of phenylephrine hydrochloride. The first method is based on a rapid azo-coupling reaction between PHE·HCl and diazotized 3,4,5-trimethoxyaniline (TMA) in an aqueous medium, as illustrated in Figure 1b. 3,4,5-Trimethoxyaniline is an important intermediate in organic synthesis and has been widely utilized in antibacterial, antitumor, and aromatic amine-related applications [29–31]. The second method involves cloud point extraction (CPE) of the azo dye formed between PHE·HCl and diazotized TMA in the presence of the non-ionic surfactant Triton X-114. The combination of spectrophotometry with CPE offers enhanced sensitivity and selectivity while serving as a practical alternative to more complex and

costly analytical techniques [32–34]. In addition, the environmental impact of the proposed methods was evaluated using the Analytical Eco-Scale to assess their analytical greenness.

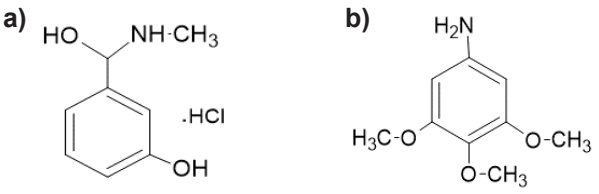


Figure 1. Chemical structures of (a) phenylephrine hydrochloride (PHE·HCl) and (b) 3,4,5-trimethoxyaniline (TMA).

Experimental part

Instruments

All spectrophotometric measurements were carried out using a JASCO V-360 UV–Vis spectrophotometer equipped with a fixed spectral bandwidth of 1 nm and 1 cm path-length glass and quartz cuvettes. Absorbance data were recorded using a connected laptop. pH measurements were performed with a TRANS BP3001 pH meter. An ABS 120-4 analytical balance (Kern & Sohn, Germany) was used for all weighing operations. Phase separation in cloud point extraction experiments was achieved using an MSE-type centrifuge.

Reagents and materials

All reagents were of analytical reagent grade and were used without further purification. Phenylephrine hydrochloride standard was supplied by the State Company for Drug Industries and Medical Appliances (SDI), Samarra, Iraq. 3,4,5-Trimethoxyaniline (Fluka), sodium nitrite (Analar), hydrochloric acid (Fisher Scientific), sodium hydroxide (BDH), Triton X-114 (Merck), and absolute ethanol were used as received. Distilled water was employed throughout the experiments.

Preparation of solutions

Phenylephrine hydrochloride standard solution (100 μg mL^{-1}). A 0.0100 g portion of phenylephrine hydrochloride was dissolved in distilled water and diluted to 100 mL in a volumetric flask. The solution was stored in an amber bottle at room temperature and was stable for one week.

3,4,5-Trimethoxyaniline solution (400 μg mL^{-1}). A 0.0400 g portion of 3,4,5-trimethoxyaniline was dissolved in 5 mL of ethanol and diluted to 100 mL with distilled water in a volumetric flask.

Sodium nitrite solution. A 0.01 M stock solution was prepared by dissolving 0.069 g of sodium nitrite in distilled water and diluting to 100 mL. A working solution of 1.1 × 10^{-3} M sodium nitrite was prepared by diluting 11 mL of the stock solution to 100 mL with distilled water.

Hydrochloric acid solution (1 M). A 1 M HCl solution was prepared by diluting 8.6 mL of concentrated hydrochloric acid (12.1 M) to 100 mL with distilled water.

Sodium hydroxide solution (1 M). A 1 M NaOH solution was prepared by diluting a 10 M sodium hydroxide stock solution with distilled water and stored in a plastic container.

Triton X-114 solution (10% v/v). A 10 mL volume of Triton X-114 was diluted to 100 mL with cold distilled water in a volumetric flask.

Pharmaceutical sample preparation

Nasal drops (100 μg mL^{-1}). An aliquot of 1.0 mL of Nazafrine® nasal drops (1%) (Safa Co., Diyala, Iraq) was transferred into a 100 mL volumetric flask and diluted to volume with distilled water.

Tablets (100 μg mL^{-1}). Five tablets of Samadol® (Samarra, Iraq), each labeled to contain 5 mg of phenylephrine hydrochloride, were finely powdered and mixed. An accurately weighed portion equivalent to 0.0100 g of phenylephrine hydrochloride was dissolved in distilled water, filtered, and diluted to 100 mL in a volumetric flask. The solution was stored in a dark bottle. The same procedure was applied to Panacol® caplets (Mass. P.I., U.A.E.).

General procedure and calibration graph

Method (A): Azo-coupling reaction

Under optimized conditions, 1.25 mL of 3,4,5-trimethoxyaniline solution (400 μg mL^{-1}) and 1.25 mL of sodium nitrite solution (1.1 × 10^{-3} M) were transferred into a series of 10 mL volumetric flasks. Then, 1.1 mL of hydrochloric acid (1 M) and appropriate volumes (0.1–2.2 mL) of phenylephrine hydrochloride solution (100 μg mL^{-1}) were added to obtain final concentrations in the range of 1–22 μg mL^{-1}. Subsequently, 2.0 mL of cetylpyridinium chloride (CPC, 1 × 10^{-3} M) and 1.0 mL of sodium hydroxide solution (1 M) were added. After 5 min, the solutions were diluted to volume with distilled water.

The absorbance of the resulting yellowish-orange azo dye was measured at 454 nm against a reagent blank. A linear calibration graph was obtained between absorbance and phenylephrine hydrochloride concentration, obeying Beer's law over the range 1–22 μg mL^{-1} with a correlation coefficient (R^2) of 0.9993. The molar absorptivity was 1.80 × 10^4 L mol^{-1} cm^{-1}, corresponding to a Sandell's sensitivity of 0.0113 μg cm^{-2}. The limits of detection (LOD) and quantification (LOQ) were 0.08 μg mL^{-1} and 0.29 μg mL^{-1}, respectively.

Method (B): Cloud point extraction (CPE)

Method (B) is based on the azo-coupling reaction described in Method (A), followed by cloud point extraction using Triton X-114. Into a series of 10 mL volumetric flasks, 1.0 mL of 3,4,5-trimethoxyaniline solution (400 μg mL^{-1}) and 1.0 mL of sodium nitrite solution (1.1 × 10^{-3} M) were added, followed by 1.1 mL of hydrochloric acid (1 M). Appropriate volumes (0.03–1.2 mL) of phenylephrine hydrochloride solution (100 μg mL^{-1}) were then added to obtain concentrations in the range of 0.3–12 μg mL^{-1}, followed by 1.1 mL

of sodium hydroxide solution (1 M) and 0.25 mL of Triton X-114 solution (10% v/v). After incubation for 5 min, the solutions were diluted to volume with distilled water, resulting in a turbid mixture.

The flasks were placed in a thermostatic water bath at 60 °C for 5 min to facilitate phase separation, followed by centrifugation at 4000 rpm for 20 min. After cooling, the aqueous phase was carefully decanted, and the surfactant-rich phase was dissolved in 1.0 mL of absolute ethanol and transferred to a 1 cm plastic cuvette. The absorbance was measured at 454 nm against ethanol as a blank.

A linear calibration graph was obtained over the concentration range 0.3–12 μg mL^{-1} with a correlation coefficient (R^2) of 0.9976. The molar absorptivity and Sandell's sensitivity were calculated as 3.73 × 10^4 L mol^{-1} cm^{-1} and 0.00545 μg cm^{-2}, respectively.

The calibration curves obtained for phenylephrine hydrochloride using methods (A) and (B) showed good linearity over the studied concentration ranges, as shown in Figure S1, *Supplementary Materials*.

Results and discussion

Principle of methods (A) and (B)

Method (A) is based on the formation of a diazonium salt through the reaction of 3,4,5-trimethoxyaniline (TMA) with an equimolar amount of sodium nitrite in an acidic medium. The resulting diazonium compound subsequently undergoes an azo-coupling reaction with phenylephrine hydrochloride (PHE·HCl) in an alkaline medium, yielding a stable yellowish-orange azo dye. The formed chromophore exhibits a maximum absorbance at 454 nm, as shown in the absorption spectrum presented in Figure 2a.

In Method (B), the azo dye produced in Method (A) is subjected to cloud point extraction using Triton X-114 as a non-ionic surfactant. Upon heating and subsequent cooling to the cloud point, a turbid dispersion forms, followed by phase separation into an aqueous phase and a surfactant-rich phase. The surfactant-enriched phase containing the analyte is then isolated and analyzed spectrophotometrically. The absorption spectrum of the extracted product also shows a maximum at 454 nm (Figure 2b), indicating that the cloud-point extraction process does not alter the azo dye's chromophore.

A comparison of the absorption spectra obtained in the absence and presence of Triton X-114 (Figure 2c) reveals a noticeable enhancement in absorbance intensity, accompanied by slight spectral changes. This behavior confirms the preconcentration effect of the cloud point extraction step and explains the higher sensitivity achieved with Method (B) compared to Method (A).

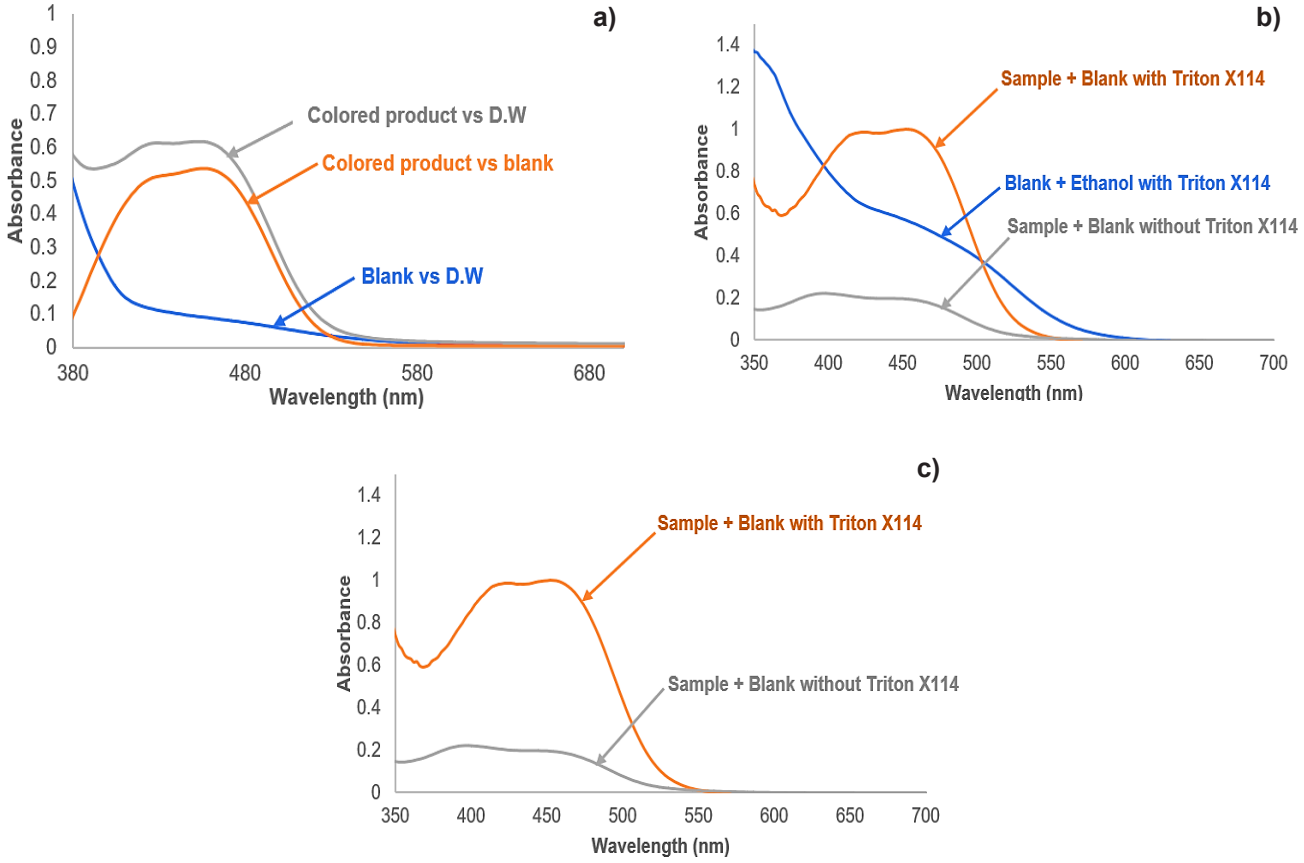


Figure 2. Absorption spectra of the yellowish-orange azo dye formed from phenylephrine hydrochloride: (a) obtained using Method (A); (b) obtained using Method (B); (c) comparison of spectra in the absence and presence of Triton X-114.

Optimum conditions of method (A)

Effect of acid type and volume

The effect of acid type on the intensity of the formed azo dye was examined using 1.0 mL of each of the following 1 M acids: hydrochloric, nitric, sulfuric, phosphoric, and acetic. Among the tested acids, hydrochloric acid produced the highest absorbance and was therefore selected for subsequent experiments. To optimize the acid volume, HCl volumes ranging from 0.25 to 2.0 mL were investigated. The maximum absorbance was obtained using 1.1 mL of HCl at pH 11.67, which was selected as the optimum volume.

Effect of reaction time

The influence of reaction time on azo dye formation was evaluated at room temperature (22 ± 2 °C). Absorbance measurements were recorded at different time intervals after the addition of sodium hydroxide. The yellowish-orange azo dye formed immediately and remained stable for at least 55 min, indicating that the reaction product remained stable throughout the measurement period.

Effect of 3,4,5-trimethoxyaniline (TMA) amount

The effect of the volume of 3,4,5-trimethoxyaniline reagent (400 μg mL^{-1}) on the absorbance of the azo dye was investigated over the range of 0.25–1.5 mL. As summarized in Table S1 (Supplementary Materials), increasing the TMA volume resulted in an increase in absorbance up to 1.25 mL, beyond which no significant improvement was observed. The highest absorbance and best linearity (R^2 = 0.9998) were achieved at 1.25 mL of TMA, which was selected as the optimum reagent volume for subsequent experiments.

Effect of type and amount of base

The influence of different bases on the absorbance of the azo dye was examined using 1.0 mL of 1 M solutions of potassium hydroxide, sodium hydroxide, sodium carbonate, sodium bicarbonate, and ammonium hydroxide. Among these, sodium hydroxide produced the highest absorbance and was therefore selected as the most suitable base. To optimize the NaOH volume, 1 M NaOH volumes ranging from 0.25 to 2.0 mL were tested. The maximum absorbance was achieved using 1.0 mL of NaOH at pH 11.86, which was adopted for subsequent experiments.

Effect of surfactant

The effect of various surfactants on the absorbance of the azo dye was investigated using cationic surfactants (cetylpyridinium chloride, CPC; cetyltrimethylammonium bromide, CTAB), an anionic surfactant (sodium dodecyl sulfate, SDS), and a non-ionic surfactant (Triton X-100). The results showed that SDS and CTAB decreased the absorbance, whereas CPC and Triton X-100 enhanced the color intensity of the azo dye. Among the tested surfactants, CPC produced the highest absorbance and was therefore selected for further experiments.

Different volumes of CPC solution (1×10^{-3} M) ranging from 0.5 to 2.5 mL were added to the reaction mixture. A volume of 2.0 mL of CPC resulted in the maximum absorbance and was selected as the optimum volume.

Effect of order of reagent addition

The effect of reagent addition order on color development was examined. The sequence of addition—3,4,5-trimethoxyaniline, sodium nitrite, hydrochloric acid, phenylephrine hydrochloride, sodium hydroxide, and CPC—was found to be optimal. Any deviation from this order resulted in a noticeable decrease in absorbance and color intensity.

Stoichiometry and mechanism

Stoichiometric ratio and stability constant

The stoichiometric ratio of the reaction product was investigated using the mole-ratio method and the method of continuous variations (Job's method) [35]. The obtained results, presented in Figure S2, *Supplementary Materials,* indicate that phenylephrine hydrochloride (PHE·HCl) reacts with diazotized 3,4,5-trimethoxyaniline (TMA) to form an azo dye with a 1:1 stoichiometric ratio.

The proposed reaction mechanism for azo dye formation is illustrated in Scheme 1. The mechanism involves diazotization of 3,4,5-trimethoxyaniline, followed by an electrophilic azo-coupling reaction with phenylephrine hydrochloride in an alkaline medium, yielding a stable-colored azo compound.

The stability constant of the resulting azo dye in aqueous solution was calculated to be 3.01×10^4 L mol^{-1}, indicating good stability of the reaction product under the experimental conditions [36].

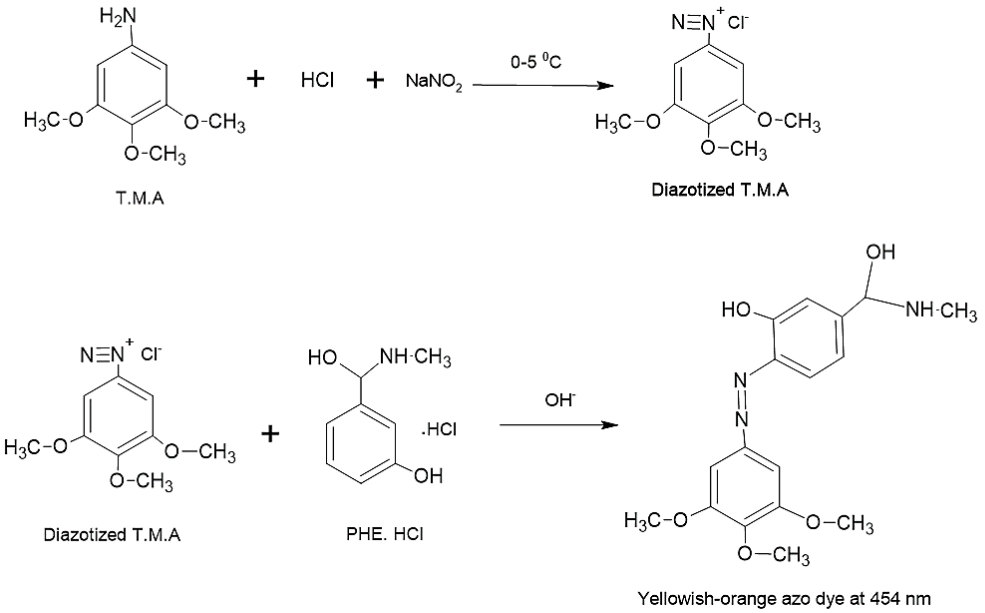
The statistical parameters and optical characteristics of the proposed spectrophotometric method are summarized in Table 2, demonstrating good linearity, high sensitivity, and satisfactory precision under the optimized experimental conditions.

Optimum conditions of Method (B)

Cloud point extraction (CPE) offers several advantages over conventional extraction techniques, including reduced consumption of organic solvents, lower toxicity, and greater environmental friendliness due to the use of non-volatile, non-flammable surfactants [37].

Effect of diazotized 3,4,5-trimethoxyaniline amount in the presence of Triton X-114

The effect of the volume of diazotized 3,4,5-trimethoxyaniline (400 μg mL^{-1}) on extraction efficiency was investigated by varying its volume from 0.25 to 1.25 mL, while keeping the volumes of sodium nitrite solution (1.1×10^{-3} M), hydrochloric acid (1.1 mL, 1 M), phenylephrine hydrochloride solution (0.2 mL, 100 μg mL^{-1}), sodium hydroxide solution (1.0 mL, 1 M), and Triton X-114 (0.5 mL, 10% v/v) constant. The results showed that 1.0 mL of diazotized 3,4,5-trimethoxyaniline provided the highest absorbance and optimal linearity at 454 nm and was therefore selected for subsequent experiments.



Scheme 1. Proposed mechanism for the formation of the azo dye between phenylephrine hydrochloride (PHE·HCl) and diazotized 3,4,5-trimethoxyaniline (TMA).

Table 1. Statistical parameters and optical characteristics of the proposed spectrophotometric method.

Parameter	Value / Condition
Linearity range (μg mL^{-1})	1–22
Molar absorptivity (L mol^{-1} cm^{-1})	1.80 × 10^4
Sandell's sensitivity (μg cm^{-2})	0.0113
Limit of detection, LOD (μg mL^{-1})*	0.08
Limit of quantification, LOQ (μg mL^{-1})*	0.29
Average recovery (%)**	101.21
Relative standard deviation, RSD (%)**	0.78
Correlation coefficient (R^2)	0.9993

* Average of ten blank determinations (n = 10); LOD = 3.3σ/S, LOQ = 10σ/S, where *S* is the slope of the calibration curve, and σ is the standard deviation of the blank ($\sigma = \sqrt{[\sum(X_i - \bar{X})^2/(n-1)]}$).
** Average of five determinations (n = 5).

Effect of Triton X-114 amount

The influence of surfactant volume on cloud point extraction efficiency was examined using different volumes (0.10–0.75 mL) of Triton X-114 (10% v/v). After the addition of 1.0 mL each of the diazotized reagent and sodium nitrite, followed by the addition of the optimized volume of phenylephrine hydrochloride solution (1.0 mL, 100 μg mL^{-1}), the mixtures were diluted to 10 mL with distilled water and incubated in a thermostatic water bath at 50 °C for 10 min.

The resulting turbid solutions were transferred to centrifuge tubes and centrifuged at 4000 rpm for 20 min, then cooled in an ice bath to facilitate phase separation. The aqueous phase was carefully decanted, and the surfactant-rich phase was dissolved in 1.0 mL of absolute ethanol. The absorbance of the extracted complex was measured at 454 nm against a reagent blank. The highest absorbance was obtained using 0.25 mL of Triton X-114, which was selected as the optimum surfactant volume.

Effect of sodium hydroxide volume

The effect of sodium hydroxide volume on the formation and extraction of the azo dye was investigated by varying the volume of 1 M NaOH between 0.5 and 1.5 mL, while keeping other experimental parameters constant. The maximum absorbance was observed at pH 10.21 using 1.1 mL of NaOH, which was therefore selected as the optimum volume.

Effect of temperature and extraction time

The influence of equilibration temperature on the cloud point extraction process was studied over the temperature range of 22–70 °C using a fixed incubation time of 5 min. The absorbance of the extracted azo dye increased with temperature, reaching a maximum at 60 °C, after which it decreased. Consequently, 60 °C was selected as the optimum equilibration temperature for all subsequent experiments.

The effect of incubation time on extraction efficiency was evaluated over the range of 5–25 min at 60 °C. An incubation time of 5 min was sufficient to achieve quantitative extraction. In addition, centrifugation times of 5-25 min were examined, and complete phase separation was achieved after 10 min. Therefore, a centrifugation time of 10 min was adopted as the optimum condition.

Precision and accuracy of methods (A) and (B)

The precision and accuracy of the proposed spectrophotometric methods were evaluated by analyzing phenylephrine hydrochloride (PHE·HCl) at two concentration levels (5 and 8 μg mL^{-1}) using both methods (A) and (B). Each concentration was analyzed in five replicate determinations. The obtained results demonstrated satisfactory precision and accuracy for both methods.

The average relative standard deviation (RSD%) was calculated using the expression RSD (%) = (S / $\overline{X}$) × 100, where S is the standard deviation, and $\overline{X}$ is the arithmetic mean. The RSD values were 2.21% and 0.775% for methods (A) and (B), respectively.

The average recovery (%) was calculated as (O / T) × 100, where O represents the observed value, and T is the true value. The recovery values obtained were 102.21% for method (A) and 101.21% for method (B), indicating good accuracy.

The average relative error (%) was calculated as [(O − T) / T] × 100 and was 2.21% and 1.21% for methods (A) and (B), respectively.

Application

The proposed spectrophotometric methods (A) and (B) were applied to the determination of phenylephrine hydrochloride (PHE·HCl) in pharmaceutical preparations, including nasal drops and tablet formulations. Standard solutions with a nominal concentration of 100 μg mL^{-1} were prepared from the pharmaceutical samples and analyzed at two concentration levels: 5 and 8 μg mL^{-1} for method (A), and 2 and 5 μg mL^{-1} for method (B), following the procedures described for the standard solutions.

The analytical results obtained for pharmaceutical formulations are summarized in Table 2. For method (A), the average recoveries were 102.48% for Samadol tablets (Samarra, Iraq), 100.71% for Panacol caplets (Mass. P.I., U.A.E.), and 101.94% for Nazafrine nasal drops (Safa Co., Iraq). For method (B), the corresponding recovery values were 101.47% and 101.24% for the tablet formulations from Samarra and Mass, respectively. P.I., respectively, and 101.04% for nasal drops.

The obtained recovery values confirm the good accuracy and efficiency of both methods for the determination of phenylephrine hydrochloride in pharmaceutical matrices. Furthermore, the calculated t-test values [38] were lower than the tabulated t value at a 95% confidence level with three degrees of freedom (N = 3), indicating no significant difference between the proposed methods and the nominal values and confirming the reliability of the methods.

Table 2. Application of the proposed spectrophotometric methods (A) and (B) for the determination of phenylephrine hydrochloride (PHE·HCl) in pharmaceutical preparations.

Drug	Method (A)					Method (B)				
	Amount μg/10 mL		Recovery * (%)	RE* (%)	RSD* (%)	Amount μg/10 mL		Recovery* (%)	RE* (%)	RSD* (%)
	Taken	Found	$t\text{-}_{exp}$ (N=3)			Taken	Found	$t\text{-}_{exp}$ (N=3)		
Sample A Samadol tablet 5 mg Samarra-Iraq	5	5.110	102.2 $t\text{-}_{exp}$=1.31	2.2	4.45	2	2.050	102.50 $t\text{-}_{exp}$=0.14	2.50	4.81
	8	8.220	102.75 $t\text{-}_{exp}$=0.39	2.75	3.01	5	5.022	100.44 $t\text{-}_{exp}$=0.28	0.44	3.25
Sample B Nazafrine Drop 1% Safa CO. Diala-Iraq	5	5.100	102.0 $t\text{-}_{exp}$=0.16	2.00	2.22	2	2.012	100.60 $t\text{-}_{exp}$=1.11	0.60	0.75
	8	8.150	101.87 $t\text{-}_{exp}$=0.73	1.87	2.26	5	5.004	100.08 $t\text{-}_{exp}$=1.31	0.08	1.47
Sample C Panacol Caplets 5mg Mass.P. I U. A. E	5	5.088	101.76 $t\text{-}_{exp}$=1.59	1.76	2.27	2	2.052	102.60 $t\text{-}_{exp}$=0.95	2.60	3.89
	8	7.972	99.65 $t\text{-}_{exp}$=0.05	-0.35	0.81	5	4.994	99.88 $t\text{-}_{exp}$= 0.79	-0.12	2.40

*Average of five determinations (n=5); t-exp^{a} : t-experimental, t-exp ± = (√N)/s ($\overline{X}$ − M).

Standard addition

The standard addition method was applied to pharmaceutical preparations to evaluate the accuracy of the proposed spectrophotometric methods (A) and (B) and to assess potential matrix interferences. Tablet and nasal drop samples were analyzed after the addition of known amounts of phenylephrine hydrochloride (PHE·HCl), following the same procedures described for the standard solutions.

The results obtained using the standard addition technique are summarized in Table 3. The satisfactory recovery values and low relative errors confirm that the proposed methods are free from significant matrix effects and are reliable for the determination of phenylephrine hydrochloride in pharmaceutical formulations.

Analytical performance and comparison with literature methods

Comparison with literature methods

The analytical performance of the proposed spectrophotometric methods (A) and (B) was evaluated and compared with previously reported methods for the determination of phenylephrine hydrochloride (PHE·HCl). A comparative summary of the main analytical parameters is presented in Table 4. As shown in Table 4, the proposed methods exhibit comparable or superior analytical performance in terms of linearity range, molar absorptivity, sensitivity, and precision when compared with related literature methods. In particular, Method (B) demonstrates enhanced sensitivity due to the incorporation of a cloud-point extraction step. An additional advantage of the proposed approaches is the absence of organic solvents, which reduce environmental and safety concerns associated with conventional extraction and separation techniques. Moreover, both methods were successfully applied to pharmaceutical preparations, confirming their practicality and reliability for routine quality control analysis.

Greenness assessment

One of the major challenges in modern analytical chemistry is developing methods that are environmentally benign, cost-effective, easy to operate, and analytically reliable. Green analytical chemistry aims to minimize the environmental impact of analytical procedures by reducing the consumption of hazardous reagents and energy, and by reducing waste generation [40]. Accordingly, the greenness of the two proposed spectrophotometric methods was comprehensively evaluated using established assessment tools [41,42].

GAPI (Green Analytical Procedure Index)

The Green Analytical Procedure Index (GAPI) is a holistic tool designed to assess the environmental impact of an analytical procedure across 15 distinct parameters, including sample preparation, reagents, instrumentation, and waste generation [42,43]. The results are visualized using five pentagram-shaped pictograms, each divided into colored segments corresponding to different stages of the analytical process. In this assessment, green, yellow, and red colors indicate low, medium, and high environmental impact, respectively.

As illustrated in Figure 3, the proposed spectrophotometric methods exhibit a favorable greenness profile when compared with the reported HPLC method [44]. The predominance of green and yellow segments reflects the use of aqueous media, the absence of toxic organic solvents, and the relatively low energy consumption associated with the proposed approaches.

Table 3. Results of the standard addition method applied to pharmaceutical preparations for the determination of phenylephrine hydrochloride (PHE·HCl) using methods (A) and (B).

Drug	Method (A)				Method (B)			
	Amount µg/10 mL		Recovery (%)	RE (%)	Amount µg/10 mL		Recovery (%)	RE (%)
	Taken	Found			Taken	Found		
Sample A Samadol tablet 5 mg Samarra-Iraq	4	3.90	97.50	-2.50	4	4.15	103.75	3.75
	7	7.16	102.28	2.28	6	6.08	101.33	1.33
Sample B Nazafrine Drop 1% Safa CO. Diala-Iraq	4	4.06	101.50	1.50	4	4.07	101.75	1.75
	7	7.06	100.86	0.86	6	6.04	100.66	0.66
Sample C Panacol Caplets 5mg Mass.P. I U. A. E	4	4.10	102.50	2.50	4	4.15	103.75	3.75
	7	6.96	99.43	-0.57	6	6.12	102.00	2.00

Table 4. Comparison of analytical performance parameters of the proposed spectrophotometric methods (A) and (B) with selected literature methods for the determination of phenylephrine hydrochloride (PHE·HCl).

Analytical parameters	Present method (A)	Literature method [27]	Present method (B)	Literature method [39]
Type of reaction	Diazo-coupling	Diazo-coupling	Cloud point extraction	Cloud point extraction
Reagent used	3,4,5-trimethoxy-aniline	2,4-dinitroaniline	3,4,5-trimethoxy-aniline	p-nitroaniline
Beer's law μg mL^{-1}	1-22	1-20	0.3 – 12	0.1 - 15
Maximum wavelength (nm)	454	455	454	591
Molar absorptivity L mol^{-1} cm^{-1}	1.80×10^4	1.915×10^4	3.73×10^4	2.75×10^4
RSD%	0.775	0.024	2.315	0.67
Sandall's sensitivity (μg cm^{-2})	0.0113	0.1063	0.00545	0.00746
Coefficient value (R^2)	0.9993	0.9985	0.9976	0.9973
Recovery %	102.21	99.97	101.21	99.7
Application	Tablets and drop	Drop and syrup	Tablets and drop	Drop

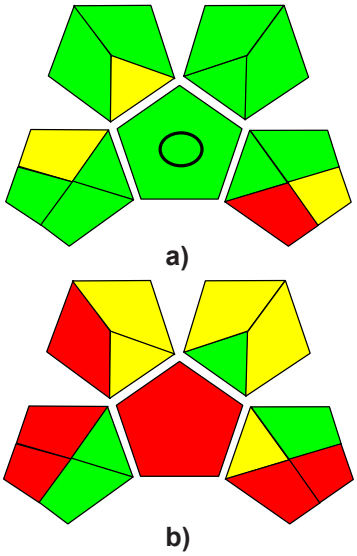


Figure 3. Analytical comparison of GAPI pictograms for (a) the proposed spectrophotometric methods and (b) a reported HPLC method [44].

Analytical Eco-scale assessment

The Analytical Eco-scale is a quantitative greenness assessment tool that evaluates the environmental suitability of an analytical method by assigning penalty points based on reagent hazards, energy consumption, instrumentation, and waste generation [45]. The Eco-scale score is calculated by subtracting the total penalty points from an ideal score of 100. An Eco-scale score of ≥85 indicates excellent green analysis; scores between 50 and 85 represent acceptable green analysis; and scores below 50 indicate insufficient greenness.

The Eco-scale penalty points for the proposed methods were calculated and compared with those of a literature HPLC method [44], as summarized in Table S2, *Supplementary Materials*. The proposed spectrophotometric approaches achieved a high Eco-scale score of 91, indicating excellent greenness, whereas the HPLC method scored 83 due to the use of organic solvents and higher energy consumption. These results confirm that the proposed methods provide a more environmentally sustainable alternative for the determination of phenylephrine hydrochloride.

Conclusion

Two simple, sensitive, and environmentally friendly spectrophotometric methods, namely a batch azo-coupling procedure and a cloud point extraction (CPE)-assisted method, were successfully developed and validated for the determination of phenylephrine hydrochloride in its pure form and in pharmaceutical preparations. Both methods demonstrated excellent analytical performance, characterized by high precision (RSD < 2.4%) and satisfactory accuracy, with recovery values ranging from 101.2% to 102.2%.

The proposed procedures eliminate the need for expensive instrumentation and hazardous organic solvents, thereby offering practical and sustainable alternatives to conventional analytical techniques. Their favorable environmental profiles were confirmed through comprehensive greenness assessment tools. Owing to their simplicity, reliability, cost-effectiveness, and eco-friendly nature, the proposed methods are highly suitable for routine quality control analysis of phenylephrine hydrochloride in pharmaceutical laboratories.

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Authors' Declaration

The authors declare that they have no competing interests regarding the publication of this manuscript.

Author's Statement

The authors certify that this manuscript represents original work and has not been published previously nor submitted for publication elsewhere. All sources of information used in this study have been appropriately cited. The authors take full responsibility for the content of the manuscript, including any errors or omissions, and confirm that all contributions have been properly acknowledged. The authors understand that any violation of these conditions may result in the withdrawal of the manuscript.

Authors' Contributions

All authors contributed equally to the conceptualization, experimental work, data analysis, and manuscript writing.

References

1. British Pharmacopoeia; *H.M. Stationery Office: London*, 2022, Vol. 3.

2. Hamed, S. L.; Qassim, B. B. A New Approach for Developing Spectrophotometric Determination of Phenylephrine Drug in Pure, Pharmaceutical and Serum Samples Using Sodium Periodate as Oxidizing Agent via a Green Method of CFIA/Merging Zone Technique. *Baghdad Sci. J.* 2024, *21* (1), 81–94. https://doi.org/10.21123/bsj.2023.7671.

3. Ahmed, H. H.; Mohammed, S. A. Spectrophotometric Estimation of Phenylephrine Hydrochloride via Oxidative Coupling Reaction with *p*-Amino Benzophenone. *Biomed. Chem. Sci.* 2023, *2* (2), 83–89.https://doi.org/10.48112/bcs.v2i2.455.

4. Boutou, E.; Virgiliou, C.; Seliniotaki, A. K.; Lithoxopoulou, M.; Mataftsi, A.; Ziakas, N.; et al. A Hydrophilic Interaction Liquid Chromatography–Tandem Mass Spectrometry Method for the Determination of Phenylephrine in Dried Blood Spots from Preterm Infants. *J. Chromatogr. B* 2022, *1190*,123084.https://doi.org/10.1016/j.jchromb.2021.123084.

5. Ahmed, H. H.; Mohammed, S. A. Development of Two Simple Spectrophotometric Methods to Assay Phenylephrine-HCl as Pure Form and in Its Drug Forms. *J. Turk. Chem. Soc., Sect. A: Chem.* 2023, *10* (3), 677–688. https://doi.org/10.18596/jotcsa.1260666.

6. Abbas, A. H.; Al-Sabha, T. N.; Al-Mtwaiti, S. M. J. Spectrophotometric Determination of Adrenaline, Phenylephrine Hydrochloride, and Terbutaline Sulfate Using the Gibbs Reagent. *J. Anal. Chem.* 2024, *79* (6), 784–797. https://doi.org/10.1134/S1061934824700163.

7. Ebrahimi, M.; Sohrab, M. R.; Motiee, F.; Davallo, M. Rapid Simultaneous Spectrophotometric Determination of Acetaminophen, Phenylephrine, and Guaifenesin in a Cold Syrup Formulation Based on Continuous Wavelet Transform and First Derivative Transform Methods. *Optik* 2021, *230*, 166323. https://doi.org/10.1016/j.ijleo.2021.166323.

8. Abdulsattar, J. O.; Hadi, H. Paper Microfluidic Device and Spectrophotometry Methods for Colorimetric Detection of Phenylephrine Hydrochloride in Pure and Pharmaceutical Preparations. *Bull. Chem. Soc. Ethiop.* 2022, *36* (4), 727–736. https://doi.org/10.4314/bcse.v36i4.1.

9. Havrylenko, O.; Kondratova, Y.; Ivanets, L.; Berdey, I.; Logoyda, L. Optimization of HPLC Methods for the Development of Quality Control Methods of Combined Powder Formulations with Paracetamol, Phenylephrine Hydrochloride, Pheniramine Maleate. *BMC Chem.* 2025, https://doi.org/10.1186/s13065-025-01488-0.

10. Barabde, G.; Pataskar, P.; Gaikar, T.; Bagul, V.; Patil, T. Development and Validation of Fast, Simple RP-HPLC Method for Simultaneous Estimation of Brompheniramine, Dextromethorphan HBr and Phenylephrine HCl in Pharmaceutical Dosage Form. *J. Appl. Chem.* 2022, *15* (8), 109–119.

11. Kelani, K. M.; Fayez, Y. M.; Abdel-Raoof, A. M.; Fekry, R. A.; Hassan, S. A. Development of an Eco-Friendly HPLC Method for the Stability Indicating Assay of Binary Mixture of Ibuprofen and Phenylephrine. *BMC Chem.* 2023, *17* (1), 1–12. https://doi.org/10.1186/s13065-023-01056-4.

12. Hamed, S. L.; Qassim, B. B. New Green Modalities of Flow Injection Technology for Assaying Anti-Allergic Drugs in Pharmaceutics and Biological Samples. *Ibn AL-Haitham J. Pure Appl. Sci.* 2023, *36* (4). https://doi.org/10.30526/36.4.3115.

13. Al-Abachi, M. Q.; Abed, S. S. Flow Injection–Spectrophotometric Determination of Phenylephrine Hydrochloride and Amoxicillin Trihydrate in Pharmaceutical Preparations. *J. Al-Nahrain Univ. Sci.* 2013, *16* (1), 42–52. https://doi.org/10.22401/jnus.16.1.07.

14. Buleandră, M.; Pătrașcu, A. A.; Popa, D. E.; David, I. G.; Badea, I. A.; Ciucu, A. A. Facile Electrochemical Sensor for Sensitive and Selective Determination of Guaifenesin, Phenylephrine, and Paracetamol on Electrochemically Pretreated Pencil Graphite Electrode. *Micromachines* 2022, *13* (8). https://doi.org/10.3390/mi13081213.

15. Rajanathan, R.; Pedersen, T. M.; Thomsen, M. B.; Botker, H. E.; Matchkov, V. V. Phenylephrine-Induced Cardiovascular Changes in the Anesthetized Mouse: An Integrated Assessment of *in vivo* Hemodynamics Under Conditions of Controlled Heart Rate. *Front. Physiol.* 2022, *13* (February), 1–12. https://doi.

org/10.3389/fphys.2022.831724.

16. Hasan, S. H.; Othman, N. S.; Surchi, K. M. Determination of Phenylephrine-HCl Using Conductometric Titration Method. *Curr. Anal. Chem.* 2015, *12* (4), 330–334. https://doi.org/10.2174/1573412912666151126205443.

17. Othman, N. S.; Abdul Fatah, N. T. Spectrophotometric Determination of Phenylephrine Hydrochloride by Coupling with Diazotized 2-Aminobenzothiazole. *Rafidain J. Sci.* 2009, *20* (4E), 69–81.

18. Al-Sabha, T. N. Spectrophotometric Assay of Phenylephrine Hydrochloride Using 4-Aminoantipyrine and Copper (II). *Jordan J. Pharm. Sci.* 2011, *4* (1), 9–19

19. Mzban, Q.; Bahjat, S.; Hassan, M. J. M. Dispersive Liquid–Liquid Microextraction and Spectrophotometric Determination of Cefazone and Phenylephrine Hydrochloride in Their Pure Forms and Pharmaceutical Preparations. *Plantarchiv.* 2020, *20* (2), 6771–6777. http://www.plantarchives.org/20-2/6771-6777%20(6664).pdf.

20. Alteemi, H. S.; Kadim, K. H. Colorimetric Determination of Phenylephrine Hydrochloride Drug by Diazotization Reaction. *J. Phys.: Conf. Ser.* 2020, *1664* (1), 012097. IOP Publishing. http://0.33899/rjs.2020.166310.

21. Ahmed, H. H.; Mohammed, S. A. Development of spectrophotometric method to assay phenylephrine hydrochloride in pure and in its pharmaceutical forms using diazotized sulphanilic acid. *Rev. Politec.* 2024, *53* (1), 39–46. https://doi.org/10.33333/rp.vol53n1.04.

22. Sharif, A. N.; Mohammed, A. A. Spectrophotometric determination of phenylephrine macromolecule by coupling with diazotized 4-bromo aniline. *Macromol. Symp.* 2025, *414* (1), 2400214. https://doi.org/10.1002/masy.202400214.

23. Hattab, A.; Ahmed, A.-M.; Anwer, S. M. Spectrophotometric determination of phenylephrine hydrochloride in pharmaceutical preparations by oxidative coupling reaction. *Int. J. Drug Deliv. Technol.* 2020, *10* (3), 323–327. https://doi.org/10.25258/ijddt.10.3.2.

24. Taha, H. K. A.; Al-Rufaie, M. M.; Motaweq, Z. Y. Utility of n-bromo succinamide for the spectrophotometric determination of phenylephrine hydrochloride in pharmaceutical formulations. *Lat. Am. J. Pharm.* 2021, *40*, 27–33.

25. Aljeboree, A. M.; Alshirifi, A. N. Determination of phenylephrine hydrochloride and amoxicillin in a binary mixture using derivative spectrophotometry methods. *Int. J. Pharm. Qual. Assur.* 2019, *10* (3), 168–177. https://doi.org/10.25258/ijpqa.10.3.10.

26. Sabr, M. W.; Ali, D. S.; Smaoui, S.; D'Amore, T. Chemometrics-assisted spectrophotometric method for the simultaneous determination of phenylephrine hydrochloride, chlorpheniramine maleate and paracetamol. *J. Pharmacol. Toxicol. Methods* 2025, *133*, 107741. https://doi.org/10.1016/j.vascn.2025.107741.

27. Othman, N. S.; Hasan, S. H.; Surchi, K. M. Using of diazotized 2,4-dinitroaniline in spectrophotometric estimation of phenylephrine hydrochloride. *Rafidain J. Sci.* 2020, *29* (3), 37–46. https://doi.org/10.33899/rjs.2020.166310.

28. Aljeboree, A. M.; Alshirifi, A. N. Colorimetric determination of phenylephrine hydrochloride drug using 4-aminoantipyrine: Stability and higher sensitivity. *J. Pharm. Sci. Res.* 2018, *10* (7), 1774–1779.

29. Xu, X.; Liu, B.; Yang, Y.; Wang, S.-F. Synthesis of 3,4,5-trimethoxyaniline. *Chem. Ind. For. Prod.* 2013, *33* (4), 93–100. https://doi.org/10.3969/j.issn.0253-2417.2013.04.018.

30. Hassan, A. H. E.; Wang, C. Y.; Lee, H. J.; Jung, S. J.; Kim, Y. J.; Cho, S. B.; Lee, C. H.; Ham, G.; Oh, T.; Lee, S. K.; Lee, Y. S. Scaffold hopping of N-benzyl-3,4,5-trimethoxyaniline: 5,6,7-

trimethoxyflavan derivatives as novel potential anticancer agents modulating Hippo signaling pathway. *Eur. J. Med. Chem.* 2023, *256*, 115421. https://doi.org/10.1016/j.ejmech.2023.115421

31. Chira, A.; Bucur, B.; Radu, G.-L. Electrodeposited organic layers formed from aryl diazonium salts for inhibition of copper corrosion. *Materials* 2017, *10* (3), 235. https://doi.org/10.3390/ma10030235.

32. Qadir, G. S.; Othman, N. S.; Al-Taee, A. T. Spectrophotometric determination of procaine hydrochloride using oxidative coupling and cloud point extraction. *Bull. Chem. Soc. Ethiop.* 2025, *39* (1), 1–13. https://doi.org/10.4314/bcse.v39i1.1.

33. Al-Saadi, M. R.; Al-Garawi, Z. S.; Thani, M. Promising technique, cloud point extraction: Technology & applications. *J. Phys.: Conf. Ser.* 2021, *1853* (1), 012064. https://doi.org/10.1088/1742-6596/1853/1/012064.

34. Mandal, S.; Lahiri, S. A review on extraction, preconcentration and speciation of metal ions by sustainable cloud point extraction. *Microchem. J.* 2022, *175*, 107150. https://doi.org/10.1016/j.microc.2021.107150.

35. Delevie, R. Principles of Quantitative Chemical Analysis, *International ed.; McGraw-Hill: Singapore,* 1997; pp 495–502. https://www.amazon.com/Principles-Quantitative-Chemical-Analysis-Robert/dp/0070163626.

36. Hargis, L. G. Analytical Chemistry: Principles and Techniques; *Prentice Hall International: London*, 1988; pp 424–427.

37. Sarsam, L. A.; Al-Sabha, T. N. Application of cloud point in spectrophotometric determination of drugs, overview. *Sci. J. Fac. Sci.-Sirte Univ.* 2024, *4* (1), 98–108. .https://doi.org/10.37375/sjfssu.v4i1.1798.

38. Christian, G. D.; Dasgupta, P. K.; Schug, K. Analytical Chemistry, 7th ed.; John Wiley & Sons, Inc.: *Hoboken, NJ*, 2014; ISBN 978-1-118-80527-5.

39. Hussein, A. M.; Othman, N. S. Determination of phenylephrine-HCl by applying cloud point extraction method using diazotized p-nitroaniline and Triton-X-114. *Alger. J. Mater. Chem.* 2022, *5*, 41–50. https://doi.org/10.5281/zenodo.7106144.

40. Saleem, K. A.; Fakhre, N.; Qader, H. A. Simultaneous determination of chlorhexidine and triclosan in some pharmaceutical personal care products using Q-absorbance ratio spectra and H-point standard addition methods. *Adv. J. Chem., Sect. A* 2024, *7* (6), 740–757. https://doi.org/10.48309/AJCA.2024.456004.1518.

41. Nowak, P.; Wietecha-Posłuszny, R.; Pawliszyn, J. White analytical chemistry: An approach to reconcile the principles of green analytical chemistry and functionality. *TrAC, Trends Anal. Chem.* 2021, *138*, 116223. https://doi.org/10.1016/j.trac.2021.116223.

42. Płotka-Wasylka, J. A new tool for the evaluation of the analytical procedure: Green analytical procedure index. *Talanta* 2018, *181*, 204–209. https://doi.org/10.1016/j.talanta.2018.01.013.

43 El-Masry, A. A.; El-Wasseef, D. R.; Eid, M.; Shehata, I. A.; Zeid, A. M. Development of three ecological spectroscopic methods for analysis of betrixaban either alone or in mixture with lercanidipine: Greenness assessment. *R. Soc. Open Sci.* 2022, *9*, 211457. https://doi.org/10.1098/rsos.211457.

44 Al-Samarrai, M. F. H.; Lafta, M. Z.; Al-Abbasee, M. J. T.; Muhammad, O. J. Development and validation of a RP-HPLC method for simultaneous quantification of paracetamol and phenylephrine hydrochloride. *Int. J. Des. Nat. Ecodyn.* 2024, *19* (1), 275–280. https://doi.org/10.18280/ijdne.190130.

45 Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. Agree-analytical greenness metric approach and software. *Anal. Chem.* 2020, *92* (14), 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887.