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Research Article

DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS USING SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Objective: A simple and sensitive spectrophotometric method has been presented for the determination of phenylephrine hydrochloride by coupling reaction with diazotized sulfacetamide sodium.

Methods: The method is based on the diazotization reaction of sulfacetamide sodium with sodium nitrite in the presence of hydrochloric acid to form diazonium salt, which is coupled with the drug in alkaline medium to form azo dye, showing absorption maxima at 425 nm.

Results: Calibration plot was linear over the concentration range of 2–24 µg/mL and detection limit of 0.278 µg/mL with a correlation coefficient of 0.9929. All different chemical and physical experimental parameters affecting on the development and stability of the colored product were carefully studied.

Conclusions: The proposed method was successfully applied to the determination of phenylephrine in nasal drops with good precision and sensitivity.

Keywords: Phenylephrine hydrochloride, Spectrophotometric determination, Sulfacetamide sodium, Diazotization and coupling.

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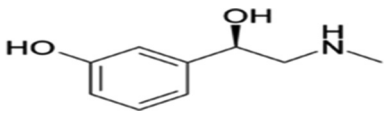
INTRODUCTION

Phenylephrine hydrochloride (PHP), [(R)-1-(3-hydroxyphenyl).2-(methylamino) ethanol hydrochloride], is a white crystalline powder, freely soluble in water, melts at 143°C [1,2] and its chemical structure is:



It belongs to a group of drugs named sympathomimetics [3]. It stimulates alpha receptors in certain areas of the body. It is used locally, as decongestant, for non-specific and allergic conjunctivitis, sinusitis, and nasopharyngitis [4]. Phenylephrine nasal drops are used for treating symptoms such as runny nose, sneezing, itching of the nose, and throat [5]. PHP is normally used to increase the blood pressure in unstable patients with hypotension, especially resulting from septic shock [5]. Various methods reported in literature for analysis of phenylephrine hydrochloride. Examples of these methods are conductometric titration [6], voltammetry [7-9], thin-layer chromatography [10], high-performance liquid chromatography (HPLC) [11-14], flow injection [15-17], and fluorescence [18]. Among the different techniques, the most popular and simple method for rapid and trace analysis of drugs is spectrophotometry [19-26].

In this work, a spectrophotometric method for estimation of phenylephrine was described. The method was based on coupling reaction between diazotized sulfacetamide sodium with the medicine in alkaline medium to form a yellow water-soluble azo dye measured at 425 nm. This method has been successfully applied for the determination of phenylephrine in nasal drops.

METHODS

Apparatus

An optima spectrophotometer ultraviolet-visible (Japan) double beam with 1 cm quartz cells was used in all absorbance measurements.

Materials and reagents

The reagent grade materials were used throughout this work. PHP, the working standard, was supplied by the State Company for Drug Industries and Medical Appliances (SDI), Samarra, Iraq. Pharmaceutical formulations (Nasophrine Nasal Drops [0.25%], SDI, Samarra, Iraq, and Vibrocil Nasal Drops [2.5 mg], Novartis Consumer Health, SA, Switzerland) were obtained from local markets. Sulfacetamide sodium (SDI, Samarra, Iraq), sodium nitrite (Merck), hydrochloric acid (HCl) (BDH), and sodium hydroxide (NaOH) (BDH) were used.

Preparation of solutions

PHP stock standard solution (1000 µg/mL) was prepared by dissolving 0.100 g of pure PHP in distilled water and made up to 100 mL volumetric flask with distilled water. Working standard solutions were prepared by suitable dilution of the stock standard solution with distilled water.

Sodium nitrite solution (3.9×10^{-3} M) was prepared by dissolving 0.0673 g of sodium nitrite in distilled water and diluting to the mark in 250 mL volumetric flask.

HCl solution (0.5 M) was prepared by diluting 10.88 mL of 11.49 M of concentrated HCl with distilled water in 250 mL volumetric flask.

Sulfacetamide sodium solution (0.1%) was prepared by dissolving 0.1 g of sulfacetamide sodium in distilled water and diluting to 100 mL volumetric flask with the same solvent.

NaOH solution (2 M) was prepared by dissolving 20 g of NaOH with distilled water in 250 mL volumetric flask.

General procedure for calibration

About 2 mL of 0.1% sulfacetamide sodium was transferred into a series of 25 mL calibrated flask. To this solution, equimolar of sodium nitrite solution (3.9×10^{-3} M) was added and the acidity was adjusted with 1 mL of 0.5 M HCl solution. The solution was shaken thoroughly. Then, an aliquot of a standard solution (500 µg/mL) containing 0.1–1.2 mL of PHP was

transferred into this series of 25 mL calibrated flasks and 1 mL of 2 M NaOH solution was added, and the contents were diluted to the mark with distilled water and mixed well. After 15 min, the absorbance of the colored azo dye was measured at 425 nm against the corresponding reagent blank. For the optimization of conditions and in all subsequent experiments, 1 mL of 500 µg/mL of PHP in a final volume of 25 mL was used.

Procedure for PHP in nasal drops

The contents of three bottles of nasal drops were mixed. An aliquot corresponding to 50 mg of PHP was diluted to 50 mL with distilled water in a volumetric flask to obtain 500 µg/mL of PHP. Further, appropriate solutions of pharmaceutical preparations were made by simple dilution with distilled water.

RESULTS AND DISCUSSION

Determination of absorption maximum

An aqueous solution of PHP is reacted with diazotized sulfacetamide sodium in alkaline medium giving yellow dye which became stable after 15 min and has a maximum absorption at 425 nm. Fig. 1 shows the spectra of the product formed.

Optimization of the experimental conditions

The effects of various parameters on the absorption intensity of the formed product were optimized.

Effect of the volume of HCl (0.5 M)

The effect of different volumes (0.3–3 mL) of HCl was examined on the maximum absorbance of the formed product. Fig. 2 shows that 1 mL of HCl (0.5 M) was enough to obtain a maximum absorbance.

Effect of the volume of sulfacetamide sodium (0.1%)

The effect of different volumes (0.5–4 mL) of sulfacetamide sodium was examined on the maximum absorbance of the formed azo dye. Fig. 3 shows that 2 mL of sulfacetamide sodium (0.1%) was enough to obtain a maximum absorbance.

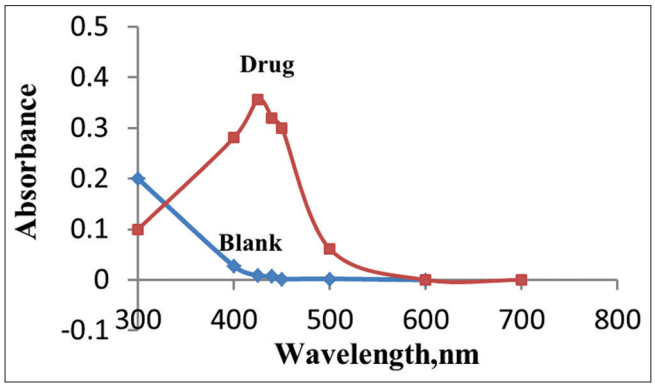


Fig. 1: Absorption spectra of the azo dye (20 µg/mL) of phenylephrine hydrochloride against reagent blank and blank against distilled water

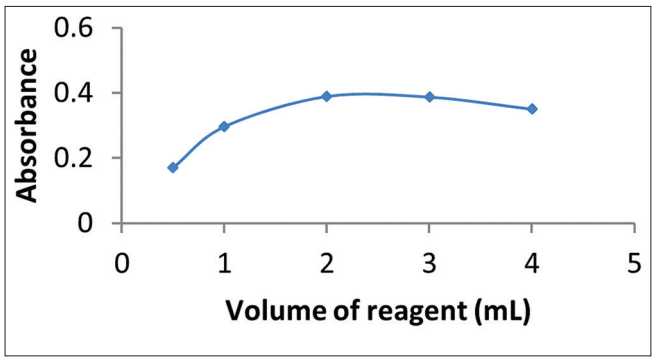


Fig. 2: Effect of the volume of hydrochloric acid (0.5 M) for the determination of phenylephrine hydrochloride (20 µg/mL)

Effect of the volume of NaOH (2 M)

The effect of different volumes (0.5–3 mL) of NaOH was examined on the maximum absorbance of the formed product. Fig. 4 shows that 1 mL of NaOH (2 M) was enough to obtain a maximum absorbance.

Effect of reaction time

The stability of the product was studied for 180 min following the mixing of the reagents. The colored product developed rapidly after mixing and attained maximum absorbance about 15 min at room temperature. The color was stable for a period of 180 min.

Structures of the products

The stoichiometry of the reaction between PHP and diazotized sulfacetamide sodium was investigated under the recommended optimum conditions using continuous variation method. The result obtained in Fig. 5 shows that a 1:2 azo dye was formed between PHP and diazotized sulfacetamide sodium.

A reaction subsequent based on the above result is shown in Scheme (1) [22].

Determination of stability constant and Gibbs free energy of the reaction

The apparent stability constant was calculated by comparing the absorbance of a solution containing 1 mL of PHP (1×10^{-3} M) and 2 mL of diazotized sulfacetamide sodium (1×10^{-3} M) (A_s) with that of a solution containing a 5-fold excess of diazotized sulfacetamide sodium (A_m) and according to analytical procedure. The average stability constant was (K) = 4.399×10^7 L^2 mol^{-2} where ($K = [1-\alpha]/4\ \alpha^3\ C^2$; $\alpha = [A_m - A_s]/A_m$) [27]. This indicates a stable reaction product. The Gibbs free energy (ΔG) of this reaction was calculated adopting the following equation: $\Delta G = -2.303RT\log K$ where, R is the universal gas constant (8.314 J $mole^{-1}$ deg^{-1}), T is the absolute temperature (273+25°C), and K is the stability constant of the reaction. The value of

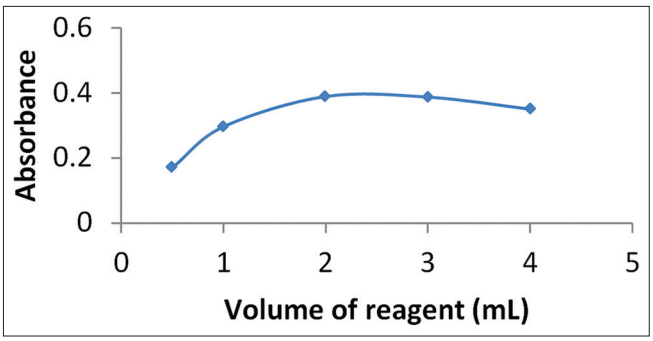


Fig. 3: Effect of the volume of sulfacetamide sodium (0.1%) for the determination of phenylephrine hydrochloride (20 µg/mL)

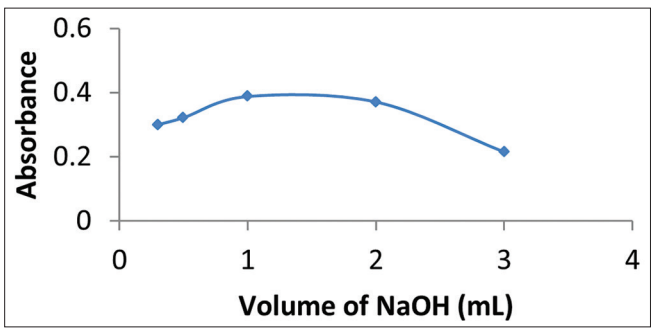
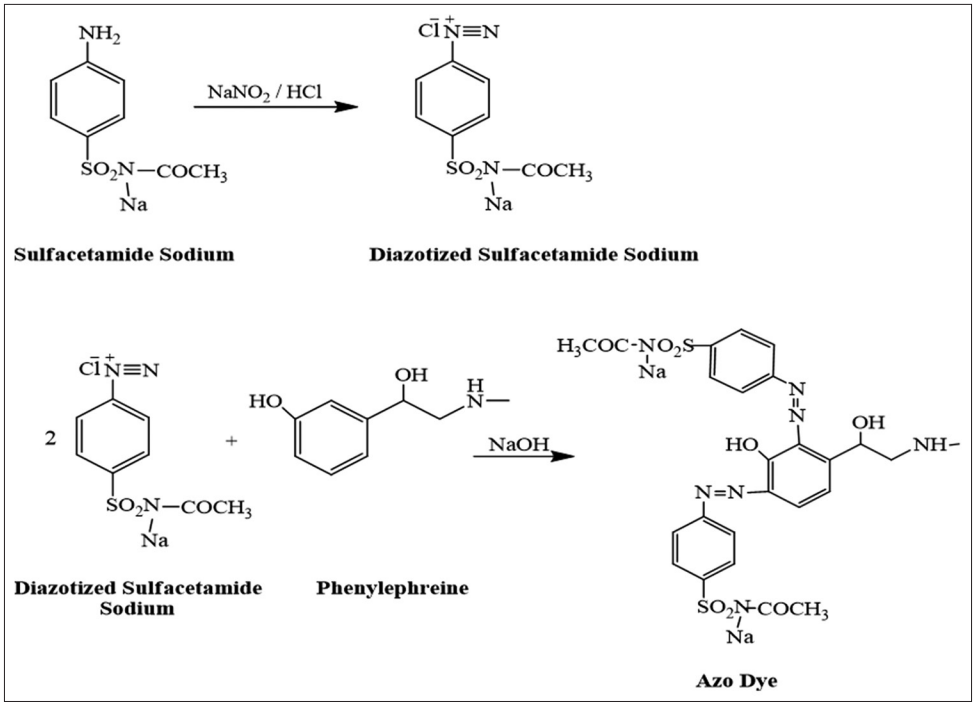


Fig. 4: Effect of the volume of NaOH (2 M) for the determination of phenylephrine hydrochloride (20 µg/mL)



Scheme 1: Proposed mechanism of the reaction between phenylephrine hydrochloride and diazotized sulfacetamide sodium

Table 1: Determination of 20 µg/mL of PHP in the presence of excipients

Excipient 200 µg/ml	Conc. of phenylephrine, 20 µg/mL (Found*)	$E_{rel.}$* (%)	Rec.* (%)
Lactose	19.917	−0.415	99.585
Starch	20.028	0.140	100.140
Talc	19.945	−0.275	99.725
Sodium chloride	20.012	0.060	100.060
Magnesium stearate	19.945	−0.275	99.725
Polyvinylpyrrolidone	19.986	0.275	100.275

*Average of four determinations, $E_{rel.}$: Relative error, Rec.: Recovery

Table 2: Analytical data obtained from the determination of PHP hydrochloride

Parameter	Value
λ_{max} (nm)	425
Beer's law limits (µg/mL)	2–24
Regression equation	Y=0.0169X+0.0084
Sandell's sensitivity (µg/mL)	5.917×10^{-5}
Molar absorptivity (L mol^{-1} cm^{-1})	3.442×10^{3}
Correlation coefficient (R^2)	0.9929
LOD (µg/mL)	0.278
Stability (min)	180
Molar ratio (D:R)	1:2
Color	Yellow

LOD: Limit of detection

Table 3: Accuracy and precision for the proposed method

Amount of PHP hydrochloride (µg/mL)		Recovery %	$E_{rel.}$ %	RSD %
Present	Found			
12.00	11.771	98.092	−1.908	1.266
16.00	15.953	99.706	−0.294	0.373

$E_{rel.}$: Relative error, RSD: Relative standard deviation

ΔG was found to be −43.612 kJ/mole. The negative value of ΔG refers to the spontaneity of the reaction.

Interferences

The extent of interfering by some excipients which often accompanied pharmaceutical preparations was studied by measuring the absorbance of solutions containing 20 µg/mL of PHP and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously (Table 1).

Analytical characteristics of spectrophotometric method

Calibration graph (Fig. 6) was obtained after optimized all the reaction conditions mentioned previously and a series of standard solutions were analyzed in triplicates to test the linearity. The molar absorptivity (ε), the Sandell's sensitivity (S), the intercept (b), and the slope (a) were determined and are included in Table 2. The limit of detection was determined by taking the ratio of the standard deviation (SD) of the blank with respect to water and the slope of the calibration curve multiplied by the factor three [28].

The accuracy and precision of the proposed method were tested by analyzing five replicate of phenylephrine by proposed spectrophotometric method for two different concentrations of phenylephrine. The values of relative SD relative standard deviation% and relative error % are summarized in Table 3. These values indicated the high accuracy and precision of the proposed method.

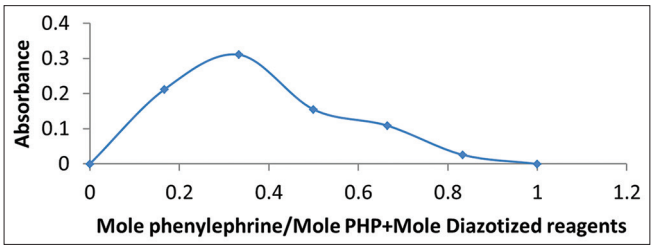


Fig. 5: Continuous variation plot

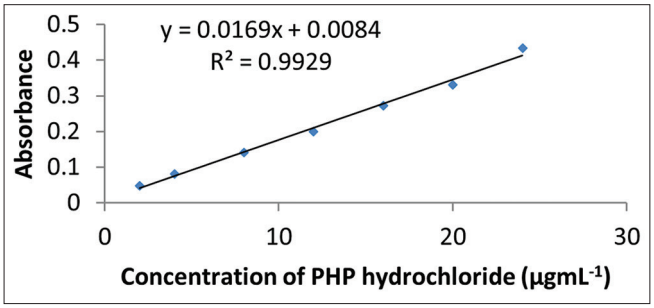


Fig. 6: Calibration graph of PHP hydrochloride

Table 4: Comparison of the proposed method with standard method for the determination of PHP in nasal drops

Pharmaceutical preparation	Recovery %	
	Proposed method	Standard method
Pure PHP	98.899	100.000
Nasophrine nasal drops (0.25%)	99.139	99.958
Vibrocil nasal drops (2.5 mg)	98.454	98.923

Pharmaceutical application

The proposed method was successfully applied to determine phenylephrine in nasal drops. The obtained results were compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with the standard method [29] at the 95% confidence level [30] as cited in Table 4. The results showed that the experimental t-test and F-test (t=1.962, F=3.079) were less than the theoretical value (F=19.00, t=2.776 ($n_1+n_2-2=4$)), indicating that there was no significant difference between the proposed method and standard method. Further, the proposed method is very economical when compared to chromatographic British pharmacopoeia methods [31].

In addition, a paired t-test [32] was conducted between the samples determined by proposed method with standard method. t-value (tab) for n-1 degree of freedom=4.303 calculated t-value=1.322 for n-1 at α 0.05 (95%), two tailed indicate that since 1.322<<4.303; therefore, it can be regarded that there is no difference between the results.

CONCLUSIONS

This research offers a simple spectrophotometric method for the determination of PHP hydrochloride in nasal drops. This method has the advantage of simplicity, speed, accuracy, and the use of inexpensive equipment. In addition, the present method, as compared with other expensive techniques such as HPLC-MS, electro-sensors, and capillary electrophoresis, is economical and cheap and has an excellent accuracy and precision.

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AUTHOR CONTRIBUTION

Wasan A. Al-Uzri: The idea of research, preparation of reagents and solutions with execution of experiments, data interpretation, and manuscript writing.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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