

Spectrophotometric Assay of Phenylphrine hydrochloride Via Coupling with Diazotised P-Nitroaniline- Application to Pharmaceutical Preparation

Abed Alghany K. Ibraheem

Department of Chemistry, College of Science, University of Tikrit, Tikrit, Iraq

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Abstract

Simple, sensitive and reproducible spectrophotometric method was developed in aqueous medium and applied for the estimation of phenylephrine hydrochloride in nasal drop and tussilet syrup. The method is based on the formation of orange coloured azo dye from the coupling of phenylephrine hydrochloride (PPH) with diazotised p-nitroaniline in alkaline medium of sodium hydroxide and sodium carbonate. The intensity of absorbance for the resulting orange azo-dye was measured at 490 nm in sodium hydroxide and at 487 nm in sodium carbonate. Beer's law was obeyed in the concentration ranges of 10-200 µg of PPH ($r^2=0.9988$) in sodium hydroxide and of 10-150 µg of PPH ($r^2=0.9981$) in sodium carbonate in a final volume of 10 ml, with molar absorptivity of 1.14×10^4 l.mol⁻¹.cm⁻¹ in sodium hydroxide and 1.98×10^4 l.mol⁻¹.cm⁻¹ in sodium carbonate. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation was +0.8 to +1.53% and relative error was -1.6 to +1.7% depending on the concentration level of (PPH), while being simple, cheap and less time consuming. The azo-dye was stable for more than 2 hours in sodium carbonate and for 13 hours in sodium hydroxide. The proposed method was successfully applied for the determination of phenylephrine hydrochloride in pharmaceutical nasal drops and tussilet syrup formulations.

التقدير الطيفي لهيدروكلوريد الفينيل فرين باستخدام الاقتران مع العامل المؤزوت بارا - نيتروانيلين - تطبيقات على مستحضرات صيدلانية
عبد الغني خليل ابراهيم

المستخلص

يتضمن البحث تطوير طريقة بسيطة وحساسة وذات استعادة جيدة في الوسط المائي لتقدير هيدروكلوريد الفينيل فرين في المستحضرات الصيدلانية (قطرة الانف وشراب التوسيليت). تعتمد الطريقة على اقتران هيدروكلوريد الفينيل فرين مع العامل المؤزوت بارا - نيتروانيلين لتكوين صبغة ازوية برتقالية اللون في وسط قاعدي من هيدروكسيد الصوديوم وكربونات الصوديوم ثم قياس شدة الامتصاص للصبغة الناتجة عند الطولين الموجيين 490 نانومتر باستخدام هيدروكسيد الصوديوم و 487 نانومتر باستخدام كربونات الصوديوم. كانت حدود تطبيق قانون بير في مدى التركيز من 10-200 مايكروغرام/10 مل من ال- PPH باستخدام هيدروكسيد الصوديوم و 10-150 مايكروغرام/10 مل من ال- PPH باستخدام كربونات الصوديوم، وقيمة الامتصاصية المولارية كانت 1.14×10^4 لتر.مول⁻¹.سم⁻¹ في هيدروكسيد الصوديوم و 1.98×10^4 لتر.مول⁻¹.سم⁻¹ في كربونات الصوديوم. تبين النتائج التي تم الحصول عليها ان الطريقة دقيقة ومضبوطة وذات استعادة جيدة، تراوح الانحراف القياسي النسبي بين +0.8 و +1.53% والخطأ النسبي بين 1.6- و +1.7% اعتمادا على تركيز ال- PPH، والطريقة بسيطة واقتصادية وتستغرق وقتا قليلا. الصبغة الناتجة مستقرة لمدة لا تقل عن ساعتين باستخدام كربونات الصوديوم و 13 ساعة باستخدام هيدروكسيد الصوديوم. تم تطبيق الطريقة بنجاح في تقدير هيدروكلوريد الفينيل فرين في قطرة للانف وشراب توسيليت للاطفال.

Introduction

Phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$, M_w $203.7 g \cdot mol^{-1}$) contains not less than 98.5% and not more than the equivalent of 101.0% of 3-(1-hydroxy-2-methylamino-ethyl)-phenolhydrochloride [(AS61-76-7)] calculated with reference to the dried

substance (Fig.1). Phenylephrine hydrochloride white or almost white, crystalline powder, freely soluble in water and in alcohol. It melts at about $143^\circ C$. the specific optical rotation is -43° to -47° , calculated with reference to the dried substance [1].

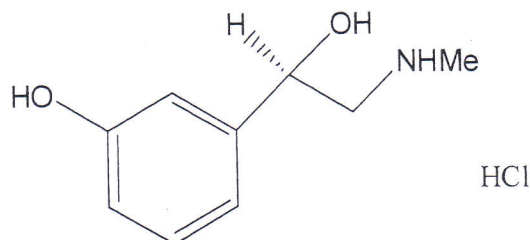


Fig. (1):- Chemical structure of phenylephrinehydrochloride

Phenylephrine hydrochloride (alpha-adrenergic, sympathomimetic agent) is a useful vasoconstrictor of sustained action with little effect on the myocardium or the central nervous system. It is available in the following dosage forms: nasal drops, nasal spray, eye drops and phenylephrine injection [2]. The use of the decongestant promotes nasal and sinus drainage. Phenylephrine is available as oral tablets, chewable tablets, oral disintegrating tablets, capsules and sachets formulations, some popular cold remedies containing phenylephrine are: Canada's hot lemon Neocitran, Serbian nasal drops, Aderianol, the United Kingdom's Lemsip, and the United States Alka-Seltzer cold, Effervescent formula, Sudafed PE Non-Drowsy Nasal Decongestant, Robitussin CF, Tylenol Sinus, and DayQuil Capsules. It is available in many combination products (with an antihistamine), such as Bromfed, Nalex-A, and Alterx. The content of phenylephrinehydrochloride is 90.0-110.0% of the stated amount [3]. It also used in the treatment of lower blood pressure (hypotension), Mydriatic of eye [4]. The important

role which is played by phenylephrine hydrochloride and its effect for the treatment of large diseases makes the assay of phenylephrine hydrochloride at most importance. Various methods have been reported in the literature for the analysis of phenylephrine hydrochloride including spectrophotometry [5-9], spectrophotometry with chromogenic reagent [10], fluorometry [11], and chromatography [12,13], high-performance liquid chromatography [14-17], micellar liquid chromatography [18], micellar electrokinetic chromatography [19], capillary zone electrophoresis [20, 21], spectro-fluorimetric and derivative spectrophotometric method [22], have also been reported for the determination of phenylephrine hydrochloride. routine analysis of phenylephrine hydrochloride, a simple and rapid analytical method is preferred. The objective of the present study was to develop simple, precise, accurate and validated, economic analytical methods for the estimation of phenylephrine hydrochloride in pure form and in pharmaceutical formulations. The present method

involves the coupling of phenylphrine hydrochloride with diazotised p-nitroaniline to form an orange dye that has been proved successfully for the assay of phenylphrine hydrochloride in nasal drop and in tussilet syrup.

Experimental

Instrument

All spectrophotometric measurements are performed on genesysTM2 UV-visible spectrophotometer by using 1cm silica cell.

Reagents

All chemicals used in this investigation are of analytical-reagent grade, and PPH standard material is provided from the general establishment for medical appliance and drugs/SDI-Samara/Iraq.

Solutions

Phenylephrine hydrochloride (PPH) solution, $100 \mu\text{g}.\text{ml}^{-1}$. This solution is prepared by dissolving 0.01g of phenylphrine hydrochloride in 20 ml of distilled water and diluting with it to a 100 ml in calibrated volumetric flask. Diazotized p-nitroaniline solution, (1mM). This solution is prepared by dissolving 0.0138g of p-nitroaniline in 50 ml hot distilled water, cooled the solution and transferred to a 100 ml calibrated volumetric flask. Add 1.5 ml of 0.8N hydrochloric acid and cooled to $0-5^{\circ}\text{C}$ by ice, after that add 0.0069g of sodium nitrite, stir the solution and after 10 minutes complete the volume to 100 ml by cold distilled water (5°C). transfer the solution to a darken brown reagent bottle and keep it in ice or in refrigerator. We can use this solution

directly after preparation and it is stable for at least seven days when it keeps in refrigerator at 5°C [23]. Sodium hydroxide, sodium carbonate solution 1N: these solutions are prepared by dissolving 4g of sodium hydroxide, 5.3g of sodium carbonate in 100 ml D.W. respectively. Sodium acetate, sodium bicarbonate solutions 1N: these solutions are prepared by dissolving 4.1g of sodium acetate, 4.2g of sodium bicarbonate in 50 ml D.W. respectively. Nasal drop solution (Sasophrine drop 0.25% PPH), $100 \mu\text{g}.\text{ml}^{-1}$: a 4ml of nasal drop solution is diluted to 100ml with D.W. Tussilet syrup solution (100 ml, 2.5mg/5ml Tussilet), $100 \mu\text{g}.\text{ml}^{-1}$: A 20 ml of tussilet syrup is diluted to 100 ml with D.W. in a calibrated volumetric flask.

Procedure and calibrations graph

To a series of 10ml calibrated flasks 3 ml of the diazotized p-nitroaniline solution, then 0.1-2 ml of $100 \mu\text{g}.\text{ml}^{-1}$ PPH solution and 0.5 ml of 1N sodium hydroxide solution or 2 ml of 1N sodium carbonate solution are added. After the volumes are completed to the mark with D.W. the absorbance is measured at 490 nm or at 487 nm respectively, against the reagent blank after 5 minutes. A linear calibration graph is obtained over the concentration range of 10-200 $\mu\text{g}/10\text{ml}$ PPH, i.e 1-20ppm (Fig.2). The molar absorptivity has been found to be $1.14 \times 10^4 \text{l}.\text{mol}^{-1}.\text{cm}^{-1}$ with sodium hydroxide and $1.98 \times 10^4 \text{l}.\text{mol}^{-1}.\text{cm}^{-1}$ with sodium carbonate.

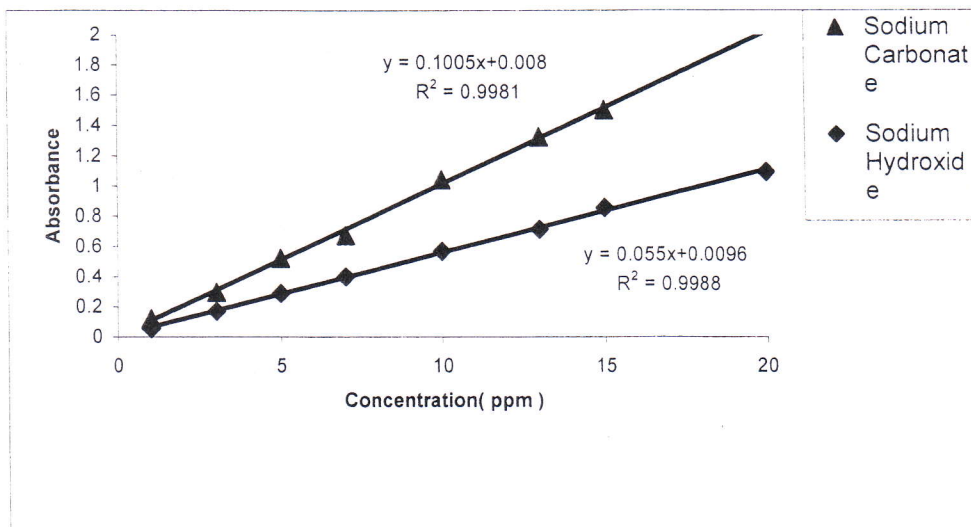


Fig. (2):- Calibration graph of PPH determination.

Results and discussion

During the investigations, a 100 µg and 50 µg of PPH is taken and the final volumes are brought to 10 ml with D.W.

Different volumes of the diazotized reagent have been used with 0.5 ml of sodium hydroxide, the results show that 3ml of (1mM) solution give the best results (table 1).

Effect of diazotized p-nitroaniline

Table 1: Effect of diazotised p-nitroaniline.

ml of diazotized P-nitroaniline	Absorbance
0.5	0.187
1.0	0.206
1.5	0.294
2.0	0.335
2.5	0.357
3.0	0.419
3.5	0.388
4.0	0.379

Effect of base

This investigation is showed that the azo-dye is formed in alkaline medium, therefore different types and amount of strong and weak bases have been studied.(table 2). The results indicate that 0.5 ml of 1N sodium hydroxide

and 2ml of 1N sodium carbonate give high intensities and high colour contrast values so they have been selected in subsequence experiments.

Table (2):- Effect of base on absorbance and colour contrast.

Base used (1N)	Variable	Absorbance/ml of base used						
		0.3	0.5	1.0	1.5	2.0	2.5	3.0
NaOH	A	0.360	0.366	0.334	0.332	0.313	0.318	0.220
Na ₂ CO ₃	A	0.390	0.474	0.487	0.518	0.555	0.532	0.526
NaHCO ₃	A	-	0.155	0.176	-	0.183	-	-

A = absorbance

Sodium acetate also studied and the results indicate that the dye which is formed decomposed quickly and show absorbance = 0.094 with 4 ml of the base.

The order of the addition:

Many experiments have been studied to know the order of the addition, the results indicate that the best order of the addition is as follows:

Diazotized agent + sample + base

The addition of the base before the sample do not form the azo-dye compound.

Effect of time

The coloured azodye is developed rapidly after addition of base and attains maximum intensity at room temperature at once. The colour is stable for at least 2 hours, till 13 hours with sodium carbonate, sodium hydroxide respectively and the results are given in table (3) and table (4).

Table (3):- The effect of time on absorbance with sodium hydroxide.

µg of PPH present	Absorbance/minute standing							
	0	5	15	20	30	40	50	13 hours
50	0.285	0.285	0.284	0.284	0.285	0.284	0.284	0.286

Table (4):- The effect of time on absorbance with sodium carbonate.

µg of PPH present	Absorbance/minute standing									
	0	5	10	20	30	45	55	110	120	125
50	0.509	0.509	0.510	0.511	0.511	0.511	0.510	0.509	0.508	0.508

Final absorption spectrum

The absorption spectrum of the orange azodye formed from coupling of diazotized p-nitroaniline with phenylephrine hydrochloride (50µg) in sodium hydroxide shows a maximum absorption at 490 nm (Fig.3) and the

orange azodye formed in sodium carbonate shows a maximum absorption at 487 nm (Fig. 4). The two reagent blanks with the two bases has no absorption at the wavelength reported.

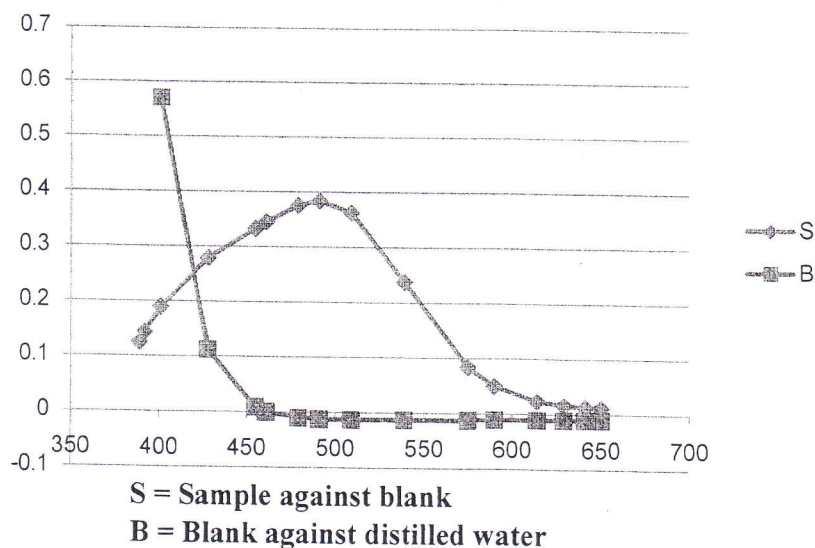


Fig.(3):- Absorption spectra of 50µg/10ml phenylephrine hydrochloride treated according to the recommended procedure and measured against (A) reagent blank, and (B) reagent blank measured against distilled water.

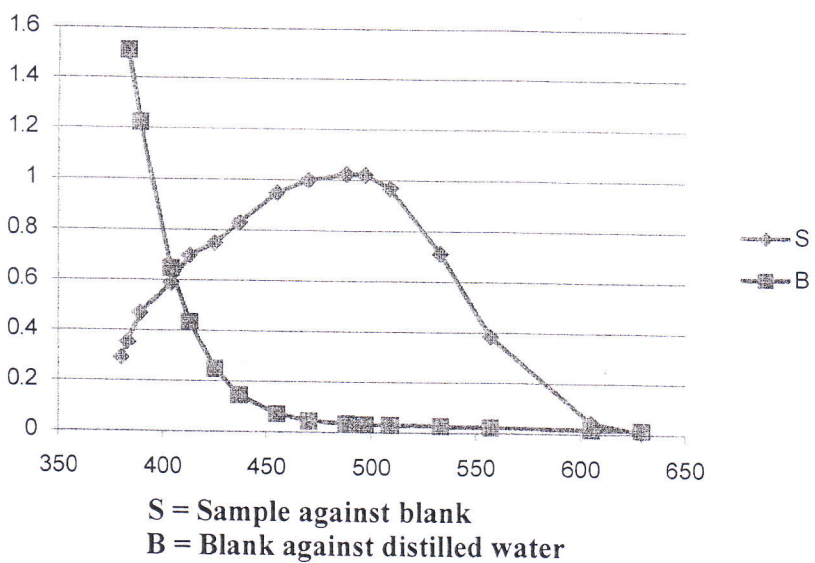


Fig.(4):-Absorption spectra of 100µg/10ml phenylephrine hydrochloride treated according to the recommended procedure and measured against (A) reagent blank, and (B) reagent blank measured against distilled water.

Nature of the azo-dye

The stoichiometry of the formed azo-dye between diazotized p-nitroaniline and phenylephrine

hydrochloride is investigated by applying Jobs method for the continuous variation[24].

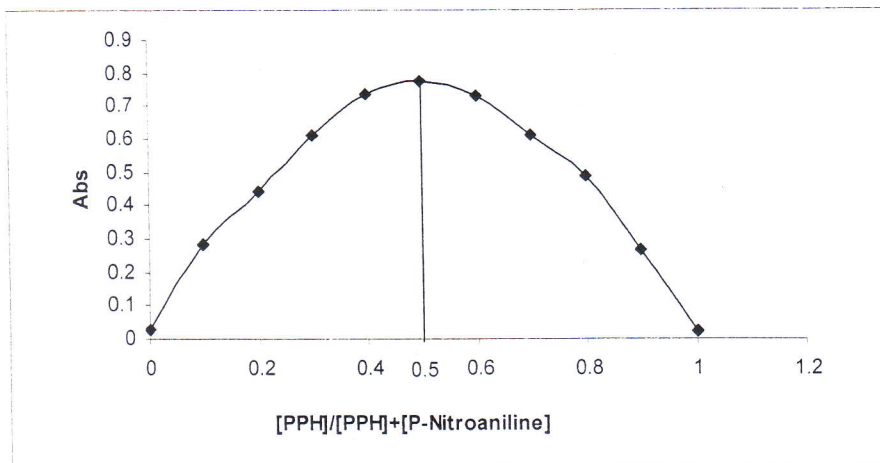
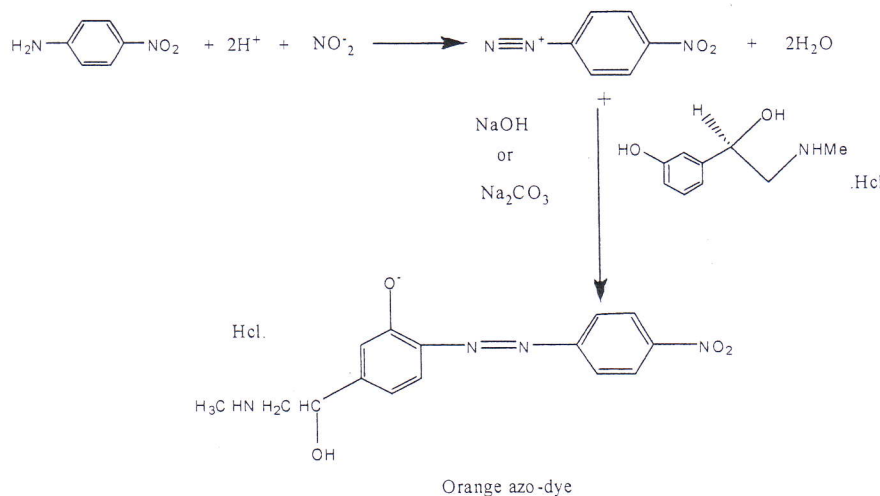


Fig.(5):-Continuous variation (Job's) plot for diazotized p-nitroaniline-phenylephrine hydrochloride azo-dye.

The results indicate that the azo-dye has been formed in the ratio of 1:1 (diazotized p-nitroaniline:

phenylephrine hydrochloride) and the azo-dye may have the following suggested structure:



Accuracy and precision

To check the accuracy and precision of present method, a three different concentrations of phenylephrine hydrochloride are determined. The

results shown in table (5), indicate that the method is satisfactory.

Table(5):- Accuracy and precision.

Amount of PPH taken $\mu\text{g}/10\text{ml}$	Relative error %	Relative standard deviation %
30	+1.1	+0.99
50	+1.7	+0.8
100	-1.6	+1.53

Analytical application

The proposed method is applied to determine phenylephrine hydrochloride in nasal drop containing 0.25% phenylephrine hydrochloride and in

tussilet syrup containing 2.5mg/5ml/phenylephrine hydrochloride. On applying proposed procedure, a good recovery is obtained (table 6).

Table(6):- Analytical applications.

Drug	Medium of coupling	Pharmaceutical preparation	Certified value	μg PPH present/ 10ml	μg PPH found/ 10ml	Recovery (%)
Nasal drop (SDI)	NaOH	Drop	0.25% PPH	30	31.2	104.0
				50	51.1	102.2
				100	97	97.0
Nasal drop (SDI)	Na_2CO_3	Drop	0.25% PPH	30	29.98	99.9
				50	50.8	101.6
				100	97.9	97.9
Tussilet syrup	NaOH	Syrup	2.5mg/ 5ml	50	51.7	103.4
				100	99.97	99.9
Tussilet syrup	Na_2CO_3	Syrup	2.5mg/ 5ml	50	50.35	100.7
				100	101.48	101.18

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