Rafidain Journal of Science
Vol. 29, No. 3, pp. 37-46, 2020

DOI : 10.33899/rjs.2020.166310

https://rsci.mosuljournals.com

ISSN: **1608-9391**
e-ISSN: **2664-2786**

Received:13/5/2020
Accepted:13/7/2020

Using of Diazotized 2,4-Dinitroaniline in Spectrophotometric Estimation of Phenylephrine Hydrochloride

Shlear H. Hasan
Department of Food Technology/ College of Agriculture/ University of Salahaddin/ Erbil/ Iraq
Nabeel S. Othman*
Department of Chemistry/ College of Science/ Mosul University
Kafia M. Surchi
Department of Chemistry/ College of Science/ University of Salahaddin/ Erbil/ Iraq

***E-mail**: nsn20002004@uomosul.edu.iq

ABSTRACT

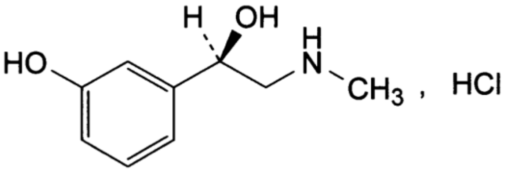
The suggested work included simple accurate diazo-coupling spectrophotometric method for the determination of phenylephrine-HCl (PHEPH) in pure drug and tablet formulation. The method was based on coupling of PHEPH with diazotized 2,4-dinitroaniline in alkaline medium. The sensitivity expressed by the value of molar absorptivity of yellowish-orange azo- dye was 1.915×10^4 L. mol^{-1}. cm^{-1}, the maximum absorption at 455 nm and Beer's law obeyed within the range of 10-200 µg/10ml of PHEPH. The color of the product was highly stable and did not show a significant change in absorbance up to 60 min. with a recovery of 99.97% and a RSD% is 0.024%. The PHEPH in nose drop and syrup has been estimated with satisfactory results.

Keywords: 2,4-dinitroaniline, phenylephrine, HCl, diazo-coupling, spectrophotometric, pharmaceutical preparations**.**

INTRODUCTION

PHEPH is structurally like to epinephrine. It is marked as an alternative for the decongestant pseudoephedrine, PHEPH is less active than pseudoephedrine and is not extra effective than placebo (Horak *et al.*, 2009). PHEPH is used for the short-term release of airless nose, and ear indications produced by the icy. This medicine works by reducing growth in the ears and nose, thus decreasing worry and creation and make it easier to breathe (Richard and Pamela, 2009).

PHEPH chemically is (R)-1-(3-hydroxyPHEPHnyl)-2- methyl amino ethanol hydrochloride, with chemical formula $C_9H_{13}NO_2$.HCl, molecular weight 203.7 g/mol., the color of crystalline powder almost white, and the solubility in alcohol and water with freely degrees. The chemical structure is shown in Scheme 1 (British Pharmacopeia, 2009).



Scheme 1. Structure of PHEPH.

The literature demonstrates that there are various analytical methods described for the estimation of PHEPHP, these methods comprised: the high performance liquid chromatography (reverse phase almost) (Parag and Rama, 2014; Sawant *et al.*, 2015; Rajan, 2015; Kalyani and Chava, 2017; Rushikesh *et al.*, 2013; Kotaiah and Kamarapu, 2013), HTLC (Wicharn and Puangkaew, 2014), conductivity (Huseen *et al.*, 2016), flow injection (Al-Abachi and Sadeem, 2013), ion pair liquid chromatography (Shanti *et al.*, 2016) and differential pulse voltammetric (Meareg and Getahun, 2017). Spectroscopic techniques remain the easy analytical methods available in our laboratories in a broader way and for the cheapest prices of devices compared to the prices of other techniques devices. Most researchers use this technique specifically the spectrophotometer in their researches published in the web to estimate PHEPH include different reactions and reagents (Anjoo *et al.*, 2017; Al-Rufaie *et al.*, 2017; Tukaram *et al.*, 2017; Wadher *et al.*, 2013; Nabeel and Abdul-fatah, 2009; Al-Abachi and Sadeem, 2015; Nabeel and Abdul-fatah, 2011; Al-Sabha, 2010; Al-Uzri, 2019; Hamoudi, 2019; Sailaja *et al.*, 2020). The aim of this study is to suggest an easy method to estimate PEHPH using diazo coupling reactions and to study the optimal conditions for estimating it in its pharmaceutical preparations.

EXPERIMENTAL

Apparatus

The absorbance and spectrum are measured and achieved by using Cecil CE3021-England UV-VIS spectrophotometer with two quartz cells (1.0 cm). Electronic balance (Sartorius AG Gottingen B2-2105 Germany), pH-meter (Korea) are also used.

Reagents and samples

All chemicals and reagents were used of analytical- reagent grade and deionized water was used throughout the investigation.

PHEPH stock standard solution, 100 µg /ml

Dissolving 0.0100 g PHEPH (provided by SDI the Company for Drug Industries and Medical Applications, Sammaria, Iraq) in 100 ml distilled water. Serial dilutions with distilled water were made to prepare working solution needed in the extraction of calibration graph.

Sodium hydroxide solution, 0.1 M.

1.0 g of sodium hydroxide (Fluka) is dissolved in distilled water in 250 ml calibrated flask and stored in a plastic bottle.

Hydrochloric acid, 1.0 M.

1MHCl was prepared by diluting 8.5 ml of concentrated acid (11.76M) to 100 ml with D.W.

Sodium nitrite solution (1%).

1.0 g of sodium nitrite (BDH) dissolved in 100 ml distilled water in a calibrated volumetric flask.

Diazotized 2,4-dinitroaniline, 5×10^{-3} M.

This solution was achieved in the above concentration by dissolving 0.2289 g of 2,4-dinitroaniline (BDH) in 1MHCl (20 ml) followed by adding 60 ml distilled water. The mixture was boiled until all 2,4-dinitroaniline was dissolved and then the solution was transferred to a 250 ml volumetric flask and let in an ice-bath (0 - 5) until the solution reached this range of temperature , 8.5 ml of 1% $NaNO_2$ was added and the mixture solution was moved strongly, then the solution was completed to the mark of flask with cold distilled water. As mentioned in literature (Nabeel and Huseen, 2015) the diazotized solution must be stored in a brown bottle in a refrigerator.

Solution of interferences, 1000 µg/ ml.

0.1 g of each interfering compound included glucose, lactose and starch dissolved in 100 ml distilled water in a calibrated flask.

Preparation of nose drop solution.

Three containers Nasal drop, Nasophrin 0.5%, have been mixed and the accurate volume (containing 0.0050 mg) of the mixture diluted to 100 ml in a calibrated flask to prepared 50 µg PHEPH / ml.

Preparation of syrup solution.

A 5 ml Tussilet syrup (2.5 mg of PHEPH in each 5 ml) was diluted to 50 ml with distilled water in a volumetric flask to get a solution 50 µg PHEPH / ml.

Suggested Procedure and Calibration Graph

To a sequence of volumetric flasks(10 ml), aliquots of PHEPH solution (100 µg /ml) were added to cover the calibration curve range (10-200) µg /10 ml. 1.0 ml of diazotized 2,4-dinitroaniline (5×10^{-3} M) reagent solution, and 1.0 ml of sodium hydroxide(0.1 M) solution were added and then volume of each flask was made up to the mark with distilled water. The maximum wavelength 455 nm was applied in determination of absorbance. Fig. (1) shows that the calibration graph was linear over the concentration from 10 to 200 µg/10 ml.

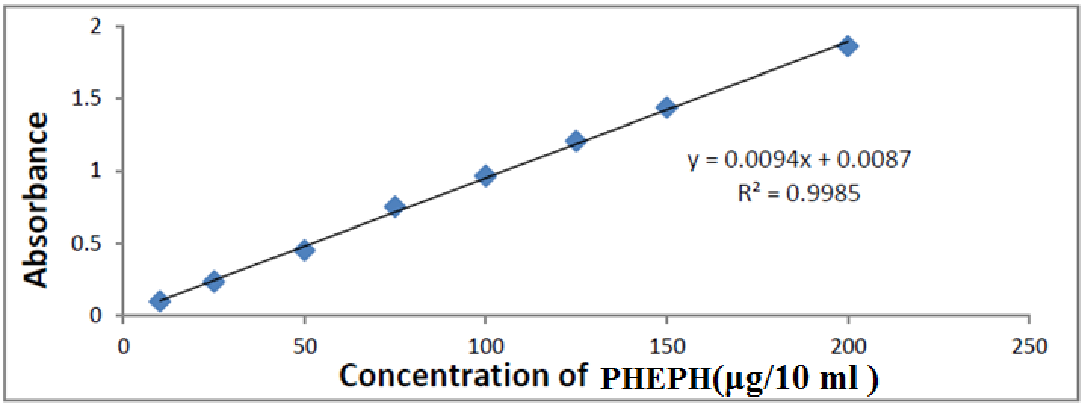
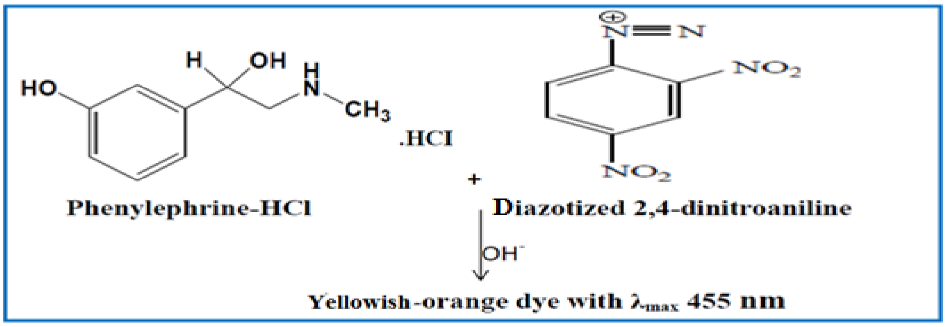


Fig. 1: The linear relationships (Calibration curve) of PHEPH determination.

RESULTS AND DISCUSSION

Principle of the Method

The procedure involved a coupling of PHEPH with diazotized 2,4-dinitroaniline reagent in basic medium to form an intensely–colored azo dye (Scheme 2).



Scheme 2: Reaction of PHEPH drug with diazotized 2,4-dinitroaniline.

Optimization of Variables

All parameters affected the color intensity of the yellow dye has been studied to stablish the optimum conditions for estimation of PHEPH via coupling it with diazotized 2,4-dinitroaniline reagent.

The selection of Diazotized Reagent

Several aromatic diazotized agents have been tested for optimum conditions. The results in Table 1 shows that diazotized 2,4-dinitroaniline reagent has been selected in this study because it is consider as the strongest diazonium electrophile compared with the other diazotized reagents in (Table 1), according to the presence of two strong electron withdrawing(nitro groups), the solution of the diazotized 2,4-dinitroaniline reagent was stable for a long time (at least one month) if it kept in cold and dark place.

Table 1: The optimum diazotized reagent

Diazotized Reagent (5×10^{-3}M)	**Absorbance**	**λ_{max}, nm**	**Δλ* nm**
4-Aminosalicylic acid	No color contrast	…..	……
2-Aminobenzoic acid	0.212	411	43
Sulphanilic acid	0.310	417	66
3-Nitroaniline	0.299	453	52
2,4-Dinitroaniline	0.514	455	105

*Δλ = Color contrast = λ_{max} S- λ_{max}B Where: S= the dye, B= blank.

Effect of diazotized 2,4-dinitroaniline Reagent Amount

The effect of different amounts of diazotized 2,4-dinitroaniline reagent on the absorbance of PHEPH (100 µg/10 ml) was studied. The obtained results indicate that the absorbance increases with increasing the reagent concentration and reaches the maximum on using a volume of 1.0 ml of 5×10^{-3} M diazotized 2,4-dinitroaniline (Table 2). Therefore, the addition of 1 ml reagent was recommended for the subsequent experiments.

Table 2: Effect of diazotized 2,4-dinitroaniline reagent amount

Volume (ml) of (5×10^{-3}M) reagent solution	Absorbance of PHEPH 100 µg/10ml
0.5	0.665
1.0	**0.831**
1.5	0.660
2.0	0.556

Effect of base type and its amount.

The primary experiment in this study shows that the yellow azo dye can be occur only in basic medium. Therefore, different amounts of various base have been examined. The results indicate that 1.0 ml of 0.1 M NaOH (pH=11.32) was selected to be optimum because it gave the highest color intensity of the azo dye formed (Table 3).

Table 3: Type and amount of optimum base

0.1 M of Base added	Absorbance / volume of base used (ml)				
	0.5	1.0	1.5	2.0	2.5
NaOH	0.112	**0.964**	0.830	0.708	0.450
KOH	0.120	0.621	0.484	0.334	0.228
Na_2CO_3	0.075	0.115	0.096	0.074	0.057
$NaHCO_3$	0.045	0.087	0.091	0.071	0.064

The stability of yellow azo dye.

The yellowish-orange azo dye appears immediately after adding the base, and after dilution there is no signified change on absorbance for at least 60 minutes (Table 4).

Table 4: The stability of formed azo dye

Time, minute	Absorbance of 100 µg PHEPH/ minute							
	0	5	10	20	30	40	50	60
Absorbance	0.964	0.963	0.964	0.966	0.967	0.965	0.965	0.966

The order of reaction components addition.

The order of addition for reagent (R) and corresponding volume of NaOH (B) to the sample solution (100 µg/10 ml of PHEPH) had been examined (Table 5). The results indicate that order (I) of addition was the best order according to the high absorbance of the yellow azo dye.

Table 5: Order of addition

Component of reaction	Order number	Absorbance
PHEPH + R+ B	I	0.967
PHEPH + B + R	II	0.415
R + B + PHEPH	III	0.660

Final Absorption Spectrum

By adding the components of reaction in their optimum amount as mentioned before an intense yellowish-orange azo dye was formed. The azo dye formed has a maximum absorption at wavelength 455 nm as shown in Fig. (2) in contrast to the reagent blank.

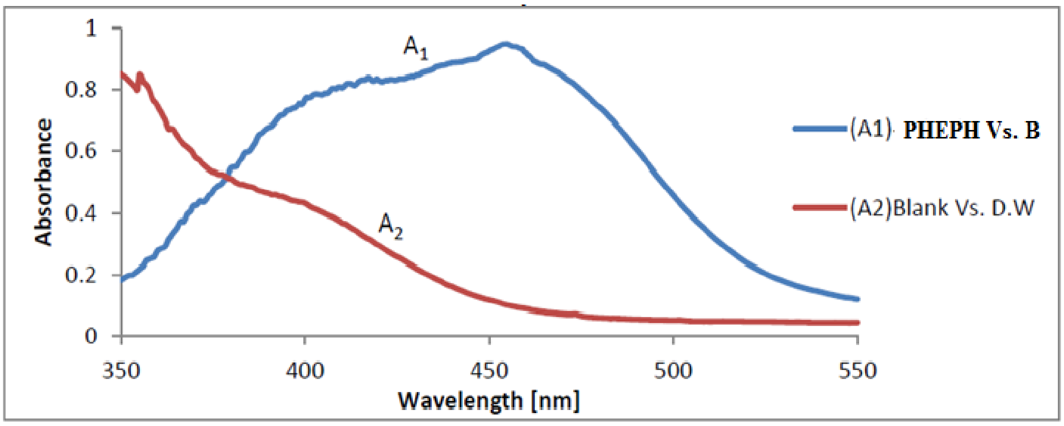


Fig. 2: Absorption spectrum of 100 µg/10 ml PHEPH (A1) the yellowish-orange azo dye measured against reagent blank (A2) blank measured against distilled water.

Accuracy and Precision

To evaluate the accuracy (recovery %) and precision (RSD %) of the calibration curve, three concentrations of pure PHEPH were analyzed in four replicates. Table (6) indicate that a satisfactory result achieved by using the proposed method.

Table 6: Accuracy and precision of proposed method

Amount of PHEPH taken (µg) in 10 ml	Amount of PHEPH found (µg) in 10 ml	Recovery*%	R.S.D %
25	24.89	99.56	0.0389
75	75.17	100.22	0.0184
150	150.21	100.14	0.015
Average		99.97	0.024

*Average of five determinations.

Stoichiometry of the Product

Job's (continuous variations) method (Job,1971) has been used to estimate the conformation of the yellowish-orange azo dye. Fig. (3) shows that the combining ratio of PHEPH and 2,4-DDNA reagent was found to be near to 1:2 ratio, the optimum positions of adding 2,4-DDNA at the ortho and para position to the hydroxyl group in the PHEPH (Nabeel and Inaam, 2005). 0.5-4.5 ml of 5 x 10^{-4} M [PHEPH] 4.5-0.5 ml of 5 x 10^{-4} M [2,4-DDNA].

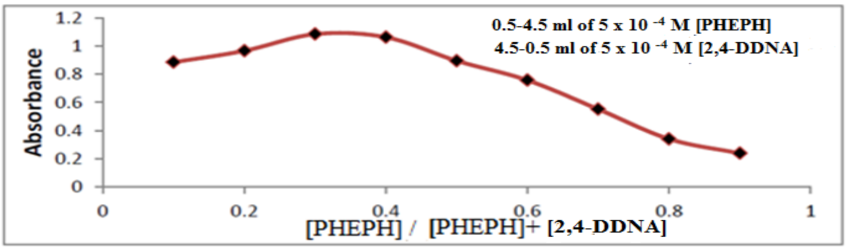
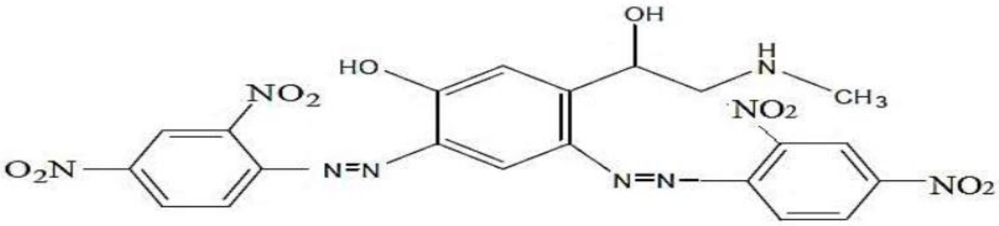


Fig.3: Job's plot for PHEPH- diazotised 2,4- dinitoaniline yellowish-

Orange Azo Dye

Therefore, the yellowish-orange azo dye structure may be suggested as follows:



Scheme 3: Yellowish-orange azo dye.

Interference Studies

The effects of common excipients used in the pharmaceutical preparations were studied by analyzing a synthetic sample solution containing 20 µg of PHEPH, in the presence of three amounts (250,500 and 1000 µg) of excipients glucose, lactose and starch. There is no interference and no change in the percentage of recovering of PHEPH up to 1000 µg of each excipient

Application of the Method

The suggested method has been applied to determine PHEPH in its pharmaceutical formulations with satisfactory results. A statistic cooperation by a student's t-test and F-test (Miller and Miller, 2005), with the standard method (British Pharmacopeia, 2009) (depending on potentiometric titration of pure drug with 0.1 M sodium hydroxide) at the 95 % confidence level with 6 degrees of freedom, as cited in (Table 7). The experimental values of t-test and F-test were less than the theoretical values, these results are demonstrated that there is no significant difference between the proposed method and the standard.

Table 7: Application of the proposed method for determination of PHEPH

Preparation	µg PHEPH present/10ml	µg PHEPH found/10 ml	Recovery% [a]	
			Present method, t and F value	Standard method
Tussilet (syrup) 2.5 mg/5 ml	100	100.76	100.76 *t* =1.07 *F* =4.81	98.92
Nasophrin (nasal drop) 0.5%	100	102.52	102.52 *t* =1.68 *F* =4.73	99.87

a-Average of 4 determinations.

Comparison of the Method

Some of the analytical variables were chosen and placed in a comparison table with their counterparts for the methods in literature. The results are shown in (Table 8).

Table 8: Analytical variables for the proposed method compared with the same for literature methods

Analytical parameters	Suggested method	Method 1 (Nabeel and Abdul-fatah,2009)	Method 2 (Hamoudi,2019)
Type of reaction	Diazo-coupling	Diazo-coupling	Diazo-coupling
Reagent	Diazotized 2,4-Dinitroaniline	Diazotized 2-aminobenzothiazole	Diazotized p-bromoaniline
λ_{max} , nm	455	510	441
Beer's law (µg/ml)	1.0-20	0.4 – 10	0.5-40
ε ($L.mol^{-1}cm^{-1}$)	1.915×10^4	6.620×10^3	4.855×10^4
Color Stability(hr.)	1	48	1
RSD%	0.024	0.95 to 3.09	…….
Applications	Nose drop and syrup	Nose drop	Tablet and syrup

Comparative results show that the proposed method is no less important than the methods used in the comparison in (Table 8) with a good value of molar absorptivity, relative standard deviation and has a wide range of application.

CONCLUSION

The suggested method is simple and sensitive, it does not involve any pretreatment of the drugs nor temperature control. The proposed method is free of interferences from the common excipients. The results obtained are closely comparable to the reported methods and recovery test is also found to be satisfactory.

REFERENCES

Al-Abachi, M. Q.; Sadeem, S. (2013). Flow injection-spectrophotometric determination of phenylephrine hydrochloride and amoxicillin trihydrate in pharmaceutical preparations. *J. Al-Nahrain University*, **16** (1), 24-24.

Al-Abachi, M.Q.; Sadeem, S.A. (2015). Spectrophotometric determination of phenylephrine hydrochloride and salbutamol sulphate drugs in pharmaceutical preparations using diazotized metoclopramide hydrochloride. *Baghdad Sci. J.*,**12**(1),167-177.

Al-Rufaie, M. M.; Jawad, A.A.; Sadiq, H. M. (2017). Colorimetric estimation for salbutamol-sulphate in pure form and in different types of pharmaceutical. *J. Pharm. Pharm. Res.*, **1** (1,1),1-7.

Al-Sabha, Th.N. (2010). Spectrophotometric assay of phenylephrine hydrochloride using 4-aminoantipyrine and copper (II). *Pak. J. Anal. Environ. Chem.*, **11**(1), 01–07.

Al-Uzri, W.A. (2019). Determination of phenylephrine hydrochloride in pharmaceutical preparations using spectrophotometric method. *Asian J. Pharm. and Clinical Rese.*, **12**(5),1-5.

Anjoo, K.; Pawan, S.; Upendra, K. (2017). Development and validation of UV spectroscopic methods for simultaneous estimation of salbutamol sulphate and doxophylline in combined solid dosage form. *Int. J. Pharmacy and Pharmaceutical Sci.*, **9** (6),118-122.

British Pharmacopeia on CD-ROM. System Simulation Ltd, the Stationary Office Ltd, London, 2009.

Hamoudi, T.A. (2019). Spectrophotometric assay of salbutamol sulphate in pharmaceutical preparations by coupling with diazotized ρ-bromoaniline. *Baghdad Sci. J.*,**16**(3), 610-615.

Horak, F.; Zieglmayer, P.; Zieglmayer, R.; Lemell, P.; Yao, R.; Staudinger, H.; Danzig M. (2009). A placebo-controlled study of the nasal decongestant effect of phenylephrine and pseudoephedrine in the vienna challenge chamber. *Ann. Allergy Asthma. Immunol.*, **102** (2), 116–120.

Huseen, S.H.; Nabeel, S.O.; Kafia, M.S. (2016). Determination of phenylephrine-HCl using conductometric titration method. *Current Anal. Chem.*,**12**,1-5.

Job, P. (1971). "Spectrochemical Methods of Analysis". Wiley Intersience, New York, 1971, 346 p.

Kalyani, L.; Chava V. (2017). Development and validation of stability-indicating RP-HPLC method for the simultaneous analysis of salbutamol, theophylline and ambroxol. *Saudi J. Med. Pharm. Sci*., **3**(11B),1265-1274.

Kotaiah, P.; Kamarapu, SK. (2013). Method development and validation of RP-HPLC method for simultaneous estimation of dextromethorphan hydrobromide, phenylephrine hydrochloride and triprolidine hydrochloride in bulk and combined tablets dosage forms. *Rese. Article Pharm. Sci*., **3**(3),172-179.

Meareg, A.; Getahun, M. (2017). Differential pulse voltammetric determination of salbutamol sulphate in syrup pharmaceutical formulation using poly (4-amino-3- hydroxyl naphthalene sulfonic acid) modified glassy carbon electrode. *Heliyon.*, **3**(10), e00417.

Miller, J.N.; Miller, J.C. (2005). "Statistics and Chemometrics for Analytical Chemistry". 5th ed., Pearson Education Limited; Harlow, pp.107-149.

Nabeel, S.O.; Abdul - Fatah, N.Th. (2011). Indirect spectrophotometric determination of phenylephrine hydrochloride in pharmaceutical preparations. *Tikrit J. Pure Sci*., **16** (2),74-81.

Nabeel, S.O.; Abdul – fatah, N., Th. (2009). Spectrophotometric determination of phenylephrine hydrochloride by coupling with diazotized 2-aminobenzothiazol. *Raf. J. Sci.,* **20**, 69-81.

Nabeel S.O.; Huseen A. M. (2015). L- thyroxine via diazo-coupling with diazotized p-nitroaniline. *Inter. J. Enhanced Res. in Sci. Tech. & Eng*., **4** (6), 290-295.

Nabeel S.O.; Hamdon I. A. (2005). Diazotised *p*-nitroaniline reagent for the determination of trace amounts of salbutamol sulphate in aqueous solution– Application to pharmaceutical preparations. *Raf. J. Sci.,* **16**(3) Chemistry, Special Issue, 60-67.

Parag, G.B.; Rama, S.L. (2014). Simultaneous determination of salbutamol sulphate and ambroxol hydrochloride in solid dosage form by RP-HPLC. *IJPRUR*., **2** (6), 408-412.

Rajan, V.R. (2015). Simultaneous determination of guaiphenesin and salbutamol sulphate in pharmaceutical dosage by reverse phase high performance liquid chromatography. *J. Chem. Pharm. Rese.,* **7**(4), 908-912.

Richard, A.; Pamela, C.C. (2009)."Lippincott's Illustrated Reviews, Pharmacology". 4th ed., New York. pp.115.

Rushikesh, B.; Atul N.; Sanjay D.S. (2013). Analytical method development and validation of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol and caffeine in bulk drug and tablet dosage form by RP-HPLC. *Indo. American J. Pharm. Rese.,* **3**(6),4330-4338.

Sailaja, B.; Venkateshwarlu, G.; Bhavani, P. N. (2020). Simple spectrophotometric method for estimation of drugs using chloraminet and indigo carmine dye couple. *Asian J. Biomed. and Pharma. Sci.,* **10**(69),19-24.

Sandhya, NK. M.; Kavitha, K. KK. (2017). A review on chromatographic methods for the simultaneous estimation of bromhexine hydrochloride and salbutamol sulphate. *Asian J. Rese. Chem. and Pharm. Sci.,* **5**(1),33 - 37.

Sawant, RL.; Bharat, AV. Tanpure, KD. Jadhav, KA. (2015). A new RP-HPLC method development for simultaneous estimation of salbutamol sulphate, theophylline and furosemide. *Inter. J. Pharm. Sci. Rese.* (IJPSR), **6** (4), ISSN: 0975-9492.

Shanti, L.S.; Sudibyo, M.; Abdul R. (2016). Validation and quantification of theophylline and salbutamol using ion pair liquid chromatography. *Indonesian J. Pharm*., **27** (4), 190 – 195.

Tukaram, M.; Kalyankar, S.J.; Mustasin, F.S. (2017). Force degraded UV-spectrophotometer method for simultaneous determination of phenylephrine HCl and bromhexine HCl in combined tablet dosage form. *Rese. Pharm.,***1**(1),06-10.

Wadher, S. J.; Kalyankar, T. M.; Panchal P. P. (2013). Development and validation of simultaneous estimation of chlorpheniramine maleate and phenylephrine hydrochloride in bulk and capsule dosage form by Ultra-Violet Spectrophotometry. *International J. Chem. Tech. Rese.,* **5**(5), 2410-2419.

Wicharn, J.; Puangkaew L. (2014). Development of HPTLC method for determination of brompheniramine maleate and phenylephrine hydrochloride tablets. *Inter. J. Pharmacy and Pharm. Sci*., **6**(6), 106-109.

استخدام الكاشف المؤزوت 4,2- ثنائي نتروانيلين في التقدير الطيفي للفينيل فرين –هيدروكلوريد

الملخص

تضمن العمل الحالي طريقة طيفية بسيطة ودقيقة لتقدير الفينيل فرين هيدروكلوريد وذلك عن طريق تفاعل الاقتران الازوي مع الكاشف المؤزوت 4,2- ثنائي نتروانيلين في الوسط القاعدي. كانت قيمة معامل الامتصاص المولاري $1.915*10^{4}$ لتر .مول$^{-1}$. سم$^{-1}$، وكان اعلى امتصاص عند الطول الموجي الاعظم 455 نانوميتر. تم تطبيق قانون بير في مدى التركيز من 10-200 مايكروغرام فينال فرين / 10 مل (1-20 مايكوغرام /مل). الصبغة المتكونة ذات استقراريه عالية ولا يوجد تغيير ملحوظ في امتصاص الصبغة ولمدة لا تقل عن 60 دقيقة، ونسبة الاستعادة والانحراف المعياري النسبي 99.97% و0.024% بالتتابع تم تطبيق الطريقة بنجاح وبنتائج مرضية في تقدير الفينيل فرين هيدروكلوريد في مستحضراته الصيدلانية (قطرة الانف والشراب).

الكلمات الدالة: فينيل فرين هيدروكلوريد، 4،2- ثنائي نيتروينلين، اقتران أزوي، الطيف الضوئي، المستحضرات الصيدلانية.