

Spectrophotometric Determination of Phenylephrine Hydrochloride by Coupling with Diazotized 2-Aminobenzothiazole

Nabeel S. Othman

Noha Th. Abdul Fatah

Department of Chemistry
College of Science
Mosul University

(Received 17 / 6 / 2009 ; Accepted 5 / 10 / 2009)

ABSTRACT

A spectrophotometric method for determination of microgram amounts of phenylephrine-HCl(PE) has been proposed. The method is based on coupling of phenylephrine-HCl with diazotized 2-aminobenzothiazole in alkaline medium. The molar absorptivity of the formed dye is $6.620 \times 10^3 \text{ l.mol}^{-1} \cdot \text{cm}^{-1}$ at $\lambda \text{ max } 510 \text{ nm}$ and Beer's law obeyed within in the range of 10-250 μg of phenylephrine-HCl /25 ml (0.4 – 10 ppm). The colour reaction was highly stable and did not show a significant change in absorbance(within acceptable analytical error) up to 48 h with a relative error +0.31 to +1.07% and a relative standard deviation of ± 0.95 to $\pm 3.09\%$, depending on concentration level. The method has been applied successfully to the determination of phenylephrine-HCl in pharmaceutical preparation (nose drop) .

Keywords: phenylephrine;2-aminobenzothiazole;dialzo-coupling; spectrophotometry

التقدير الطيفي للفنيل افرين هيدروكلورايد بالاقتران مع العامل المؤزوت 2-امينوبنزوثايزول

الملخص

تم اقتراح طريقة طيفيه لتقدير كميات متناهية في الصغر من الفنيل افرين هيدروكلوريد. تعتمد الطريقة على الاقتران مع العامل المؤزوت 2-امينوبنزوثايزول في الوسط القاعدي. بلغت قيمة معامل الامتصاص المولاري للصبغة المتكونة $6.620 \times 10^3 \text{ لتر.مول}^{-1} \cdot \text{سم}^{-1}$ عند الطول الموجي الأعظم 510 نانوميتر. وتتبع الطريقة قانون بير في مدى التراكيز من 10 - 250 مايكروغرام/ 25 مل (0.4 - 10 جزء/مليون). يمتاز التفاعل اللوني باستقرارية عالية ولا يوجد تغيير ملحوظ في الامتصاصية (ضمن الخطأ المسموح) لمدة لاتقل عن 48 ساعة وبخطأ نسبي يتراوح بين 0.31 و 1.07% وانحراف قياسي نسبي بين ± 0.95 و $\pm 3.09\%$ اعتمادا على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير الفنيل افرين هيدروكلوريد في مستحضره الصيدلاني (قطرة الأنف) .

INTRODUCTION

Phenylephrine hydrochloride[3-(hydroxyphenyl)-2-(methylamino)ethanol] hydrochloride is widely used as a decongestant drug (Al-Abachi and Al-Ward, 2002). The drug is available as an oral medicine or as a nasal spray. Phenylephrine is rarely used as a vasopressor due to its increase in the blood pressure for unstable patients with hypotension. (Louis, 1985) .

Different spectrophotometric methods have been applied for the determination of phenylephrine with different reagents such as 4-aminoantipyrine in presence of periodate or alkaline ferricyanide (Al-Abachi and Al-Ward, 2002; Hiskey and Levin, 1960) , nitrous acid in presence of copper ion (Yahia and Laila, 1976) , chloranil (Amer *et al.*, 1982) , ninhydrin in sulphuric acid (Muszalska *et al.*, 2000), periodate (Neil and Gelenn, 1971), methylbenzothiazoline-2-one hydrazone in presence of iron (Gala *et al.*, 1994), haematoxylin (Ibrahim;Alaa,2007), 4-aminophenol (Sane and Narkar,1980), chloramine (Szekerer *et al.*, 1973), N,N-dimethylaniline hydrochloride with potassium ferricyanide (Tatsuzawa and Shimoda, 1968), hydroxyl ammonium chloride with cupric ion (Deodhar and Mehta, 1978), sodium borate (Doulakas, 1975), fluorodinitrobenzene (Tammilehto, 1975), bromothymol blue (Matthew *et al.*,1972), and diazotized p-nitroaniline (Auerbach, 1950; Kelly and Auerbach, 2006). Also, the ultraviolet (Fabrizo, 2006) and derivative spectrophotometry (Kazemipour and Ansari, 2005) methods have been used.

The flow injection methods have been used in the determination of phenylephrine with spectrophotometric detection (Yolanda *et al.*, 2001; Knochen and Giglio, 2004; Beyene and Vanstaden, 2004).

Also, the high performance liquid chromatography technique has been applied to the determination of trace amounts of phenylephrine (Amer *et al.*, 2008; Marin and Barbas, 2004, Senyuva and Ozden, 2002).

The objective of investigation reported in this paper is to evaluate a sensitive and an accurate method for the assay of phenylephrine in an aqueous medium, either in pure form or in its pharmaceutical preparations. The method based on coupling of phenylephrine with diazotized 2-aminobenzothiazol in alkaline medium to produce an intense azo dye which was considered desirable to develop an additional assay method suitable for accurate and reliable quality control of phenylephrine formulations.

EXPERIMENTAL

Apparatus

Shimadzu UV-Visible Recording Spectrophotometer UV-160 with 1.0 cm matched silica cells was used for all absorption measurements.

Reagent

All Chemicals used were of analytical-reagent grade .

A pure phenylephrine - HCl (PE) was obtained from the State company for Drug Industries and Medical Applicances(SDI), Sammara, Iraq. A solution of $100 \mu\text{g ml}^{-1}$ was prepared by dissolving 0.01g of phenylephrine-HCl in 100 ml distilled water. Sodium hydroxide (2N) and different interferences solution($1000 \mu\text{g ml}^{-1}$) were prepared by dissolving the proper amount in distilled water.

The diazotized 2-aminobenzothiazole(25 mM) solution was daily prepared by dissolving 0.1877 g of 2-aminobenzothiazole (Fluka) in 10 ml ethanol and 5 ml of concentrated sulphuric acid (Samir and Uma, 2005), followed by the addition of about 25 ml distilled

water. Finally the mixture was transferred to a 50 ml volumetric flask and is cooled at 0-5 °C in an ice-bath. A 0.0862 g sodium nitrite was added and the mixture was stirred vigorously. After 5 minutes, the solution was made up to 50 ml with cold distilled water. The solution was kept in a brown bottle in a refrigerator for 1 hour before using.

General procedure and calibration graph

The aqueous solution (0.1 – 3ml) contain phenylephrine-HCl ($100 \mu\text{g} \cdot \text{ml}^{-1}$) was transferred to 25 ml calibrated flasks. A 2.5 ml of 2-aminobenzothiazole diazotized solution (25mM), and 4 ml of sodium hydroxide solution (2N) were added and the volume was made up to the mark with distilled water. The absorbance was measured at 510 nm against a blank solution which was prepared in a similar way but without the addition of phenylephrine-HCl .

The calibration graph as shown in Fig. (1) was linear over the range of 10-250 μg of phenylephrine-HCl /25 ml (0.4 – 10 ppm). Higher concentrations show a negative deviation from Beer's law . The apparent molar absorptivity referred to phenylephrine – HCl has been found to be $6.620 \times 10^3 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

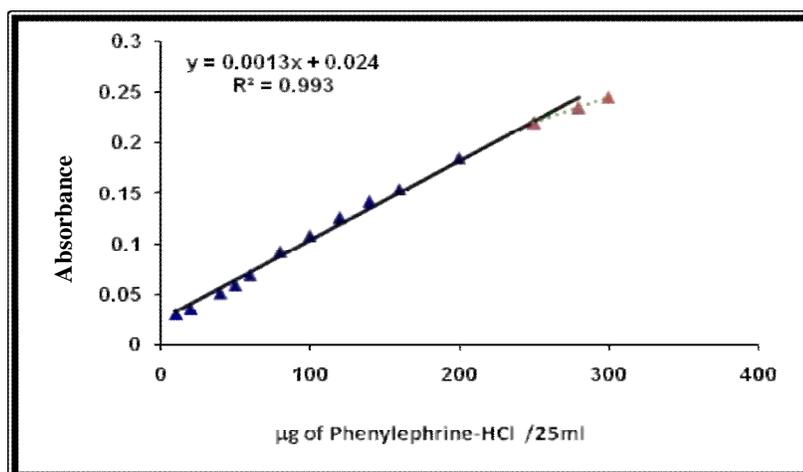


Fig. 1: Calibration graph for phenylephrine – HCl determination using the proposed method

Procedure for the phenylephrine-HCl nose drop

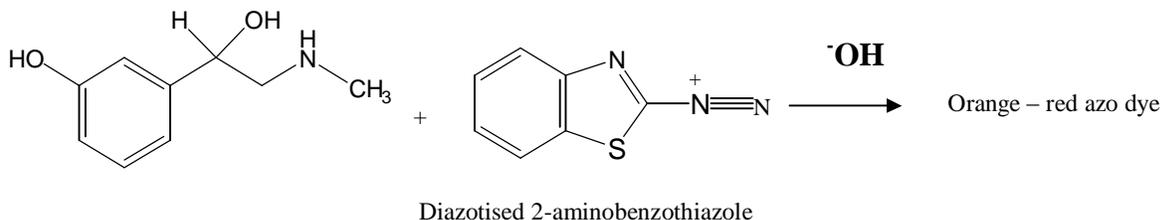
The contents of three of phenylephrine -HCl nose drop containers (nasal drop, SDI, Sammara, Iraq) were mixed. An accurate volume containing 0.005 mg phenylephrine - HCl was transferred to a 50 ml volumetric flask, and the volume adjusted to the mark with distilled water and , then it was proceeded as described under procedure for calibration.

RESULTS AND DISCUSSION

The effect of various variables on the colour development was tested to establish the optimum conditions for determination of PE by coupling with diazotized 2-aminobenzothiazole reagent.

PRINCIPLE OF THE METHOD

The method involves the coupling of phenylephrine drug with diazotized 2-aminobenzothiazole in basic medium to form an intensely – coloured azo dye:-



Choice of diazotised agent

Several aromatic diazotised agents have been tested for optimum conditions. The results in Table 1 show that 2-aminobenzothiazole give the most sensitive reaction ($\epsilon = 8.810 \times 10^3 \text{ l.mol}^{-1} \cdot \text{cm}^{-1}$) in alkaline medium. Therefore, it has been selected for subsequent experiments.

Table 1: The selection of diazotized agent

Reagent (0.025M)	Structure	$\Delta\lambda$, nm	ϵ ($\text{l.mol}^{-1} \cdot \text{cm}^{-1}$)
4-aminosalicylic acid			No colour contrast
Benzocaine		60	8.606×10^3
2-aminobenzothiazole		213	8.810×10^3
p-aminobenzoic acid		64	6.264×10^3
Sulphanilic acid		43	7.435×10^3
2-aminobenzophenone		63	5.092×10^3

* $\Delta\lambda = \text{Colour contrast} = \lambda_{\text{max}} S - \lambda_{\text{max}} B$
 where S=The dye, B=Blank

Effect of diazotized 2-aminobenzothiazole reagent amount

The effect of different amounts of diazotized 2-aminobenzothiazole reagent on the absorbance of solutions containing different amounts of phenylephrine-HCl (20-200 $\mu\text{g}/25$ ml) was studied. The obtained results indicated that the absorbance increases with increasing reagent concentration and reached a maximum on using a volume of 2.5 ml of 25 mM diazotized 2-aminobenzothiazole which also gives the highest value of coefficient of determination (r^2) (Table 2). Therefore, the addition of 2.5 ml reagent was recommended for the subsequent experiments.

Table 2: The effect of the amount of diazotized 2-aminobenzothiazole on dye absorbance

ml of 2- Aminobenzothiazole solution 25 mM	Absorbance / μg of phenylephrine-HCl in 25 ml						r^2
	20	50	80	100	150	200	
1	0.025	0.030	0.040	0.045	0.074	0.075	0.93698
2	0.029	0.036	0.053	0.065	0.089	0.115	0.9935
2.5	0.029	0.058	0.090	0.103	0.134	0.170	0.9973
3	0.034	0.041	0.073	0.099	0.118	0.167	0.9749

Effect of base

Preliminary experiments have shown that PE gives coloured dye with diazotized 2-aminobenzothiazole only in basic medium. Different bases (strong and weak) have been used and the results (Table 3) indicate that the formation of the coloured dye needs a strong basic medium. Therefore 4 ml of 2N NaOH solution has been recommended for the subsequent experiments.

Table 3: The effect of base on dye absorbance

Base used (2N) solution	Variable	Absorbance* / ml of base used				pH range
		3	4	6	8	
NaOH	A	0.054	0.113	0.101	0.105	2.36-13.32
	$\Delta\lambda^{**}$, nm	139	232	233	230	
KOH	A	0.045	0.052	0.086	0.088	2.34-13.65
	$\Delta\lambda$, nm	139	137	228	226	
Na ₂ CO ₃	A	0.030	0.029	0.026	0.029	1.82-9.70
	$\Delta\lambda$, nm	62	63	61	61	
NaHCO ₃	A	0.001	0.022	0.170	0.191	1.36-7.10
	$\Delta\lambda$, nm	136	129	78	58	

* Adding 2.5 ml of diazotised 2-aminobenzothiazole

** $\Delta\lambda = \lambda_{\max}S - \lambda_{\max}B$, where S=The dye, B=Blank

Effect of surfactant

The effect of several types of surfactants on colour intensity of the dye has been investigated. The results indicate that addition of surfactants give no useful effect [increasing the intensity or improving the colour contrast ($\Delta\lambda$)], therefore it has not been used in the subsequent experiments.

Order of addition of reagents

The order of additions of reagents [phenylephrine-HCl (PE), NaOH(OH), 2-aminobenzothiazole (R)] was examined. The results (shown in Table 4) indicated that order (I) of addition of reagents was the optimum order due to the high intensity of the formed azo dye.

Table 4: Effect of order of addition

Reaction component	Order number	Absorbance
PE + R+OH	I	0.109
OH + PE + R	II	0.075
OH + R + PE	III	0.014

Effect of time and amount of PE on absorbance

The effect of time on the development and stability period of the formed coloured dye was investigated under optimum experimental conditions described before. The formation of coloured dye being complete after mixing the component of reaction and the absorbance of the coloured species remained constant for, at least 48 hours (Table5).

Table 5: The effect of time on absorbance

Time/min.	Absorbance/ μg of phenylephrine-HCl		
	100	200	260
0	0.085	0.172	0.301
5	0.084	0.171	0.301
10	0.084	0.171	0.301
20	0.083	0.171	0.301
30	0.084	0.172	0.301
40	0.084	0.172	0.301
50	0.084	0.172	0.301
60	0.084	0.172	0.301
120	0.089	0.172	0.300
18h	0.085	0.171	0.299
24h	0.086	0.170	0.292
48h	0.082	0.166	0.290
72h	0.080	0.162	0.284
96h	0.079	0.162	0.292

Final absorption spectra

An absorption spectra of the formed coloured dye by coupling of PE with diazotized 2-aminobenzothiazole in basic medium, against its corresponding reagent blank show a maximum absorption at 510 nm in contrast to the reagent blank (Fig 2).

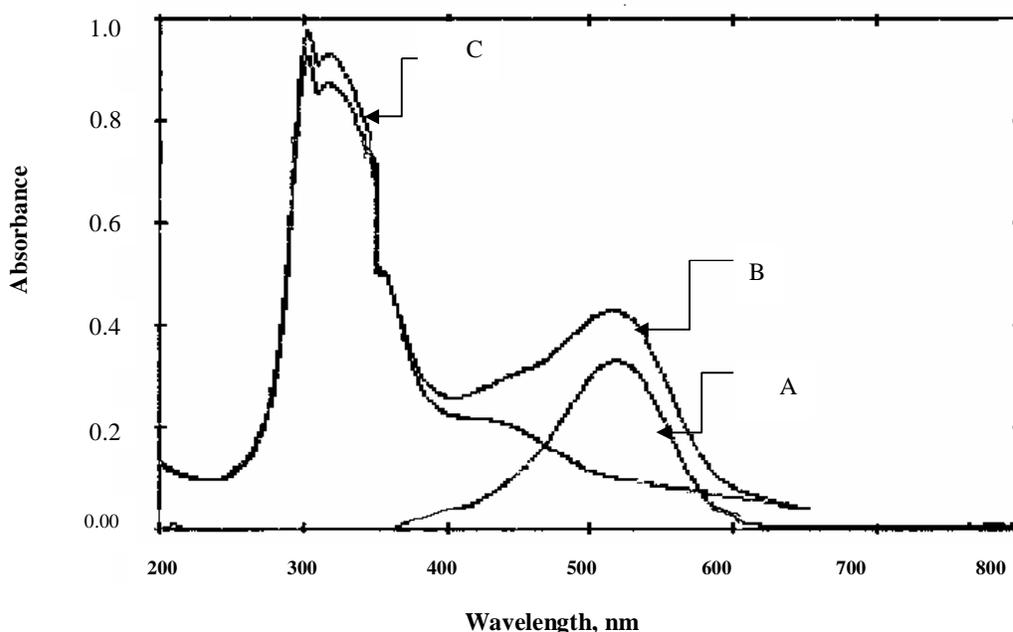


Fig. 2: Absorption spectra of 250 µg PE treated according to the recommended procedure and measured against (A) blank (B) distilled water and (C) blank measured against distilled water.

Interference

The criterion of interference was an error of not more than $\pm 5.0\%$ in the absorbance. To test the efficiency and selectivity of the proposed analytical method, a systematic study of excipients (e.g., glucose, lactose, gum Arabic and starch) that usually present in dosage forms was performed. Experimental results showed that there was no interference from additives or excipients up to 1000 µg in the present method as shown in Table 6.

Table 6 : Effect of foreign compounds for assay of phenylephrine-HCl

Foreign compound	Recovery (%) of 100 µg phenylephrine-HCl per µg foreign compound added		
	100	500	1000
Glucose	101.6	100.8	103.3
Gum Arabic	94.1	105	96.6
Lactose	107.5	103.3	97.50
Starch	105.8	100.8	100.8

Accuracy and precision

To check the accuracy and precision of the calibration curve, PE was determined at three different concentrations. The results (illustrated in Table 7) indicate that the method is satisfactory.

Table 7. Accuracy and precision of the calibration curve

Amount of phenylephrine-HCl taken, μg	Relative error, %*	Relative standard deviation, %*
80	+1.07	± 3.09
160	+0.74	± 1.21
260	+0.32	± 0.95

*Average of four determinations

Nature of the Dye

Job's and mole – ratio methods (Hargis, 1988) indicate that the azo dye has a composition of 1:2 phenylephrine [PE] to diazotized 2-aminobenzothiazole [AM] reagent (Fig.3 and 4).

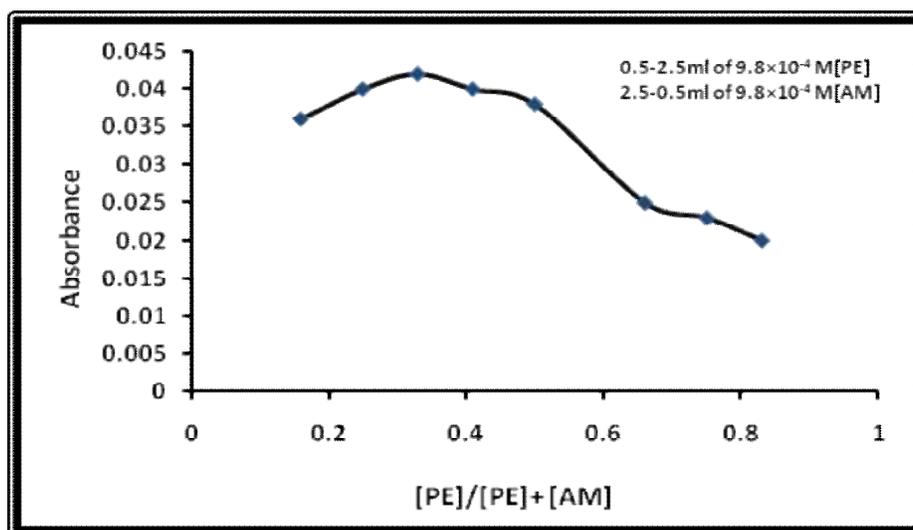


Fig.3: Job's plot for phenylephrine - diazotized 2-aminobenzothiazole

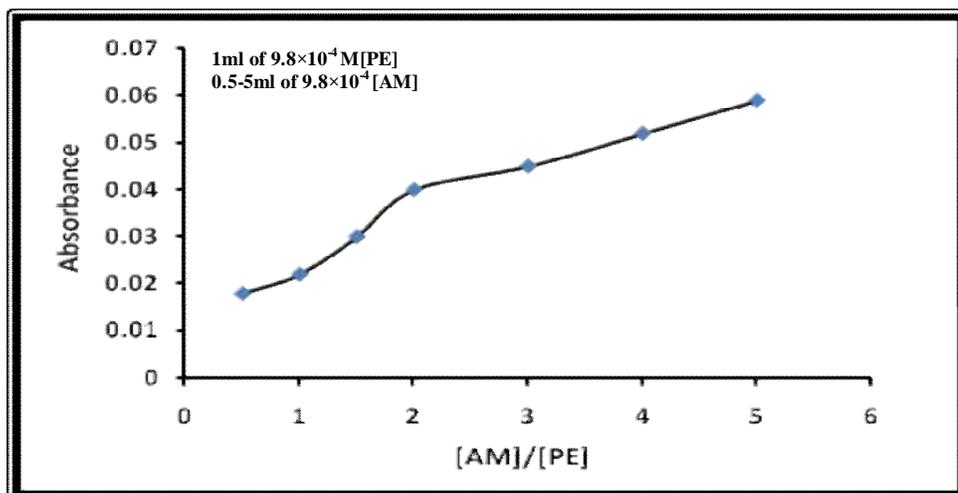
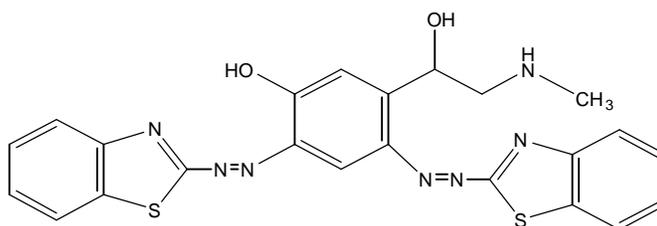


Fig.4: Mole ratio plot for- phenylephrine - diazotized 2-aminobenzothiazole

Hence, the dye may have the following structure.



Orange-red azo dye

Application of the Method

The proposed method was successfully applied to determine phenylephrine in its pharmaceutical preparation (nose drop) (Table 8). The performance of the proposed method was assessed by calculation of the t-test compared with the standard method (British Pharmacopeia, 2000) (potentiometric titration with sodium hydroxide) for 95% confidence level with four degrees of freedom. The results showed that the t-value was less than the critical value, indicated that there was no significant difference between the proposed and standard method for phenylephrine.

Table 8: Analytical applications of the proposed method and experimental t-value

Pharmaceutical preparation	μg Phenylephrine-HCl present	Recovery% by the present method	Recovery % by the standard method	t-value
Nasophrine Nasal Drop(0.25%) SDI - Iraq	200	103	99.6	1.21

Comparison of the methods

Table(9) shows the comparison between some of analytical variables for the present method with that of another literature spectrophotometric method.

Table 9: comparison of the methods

Analytical parameters	Present method	Literature method*
pH	12.97
Temperature ($^{\circ}\text{C}$)	Room temperature	Room temperature
λ_{max} (nm)	510	500
Reagent	2-aminobenzothiazole	4-aminoantipyrine
Beer's law range (ppm)	0.4-11.2	1-36
ϵ ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	0.662×10^4	1.26×10^4
Stability(hrs.)	48	1:30
Application of the method	Nose drop	Nose drop

* Al-Abachi, M. Q. and Al-Ward, H. S. National J. Chem., 2002, 6, 221.

The results indicate that the proposed method is less sensitive than the literature method but the colour is much more stable.

CONCLUSION

The proposed method was a simple and has a good sensitivity. The proposed method has advantageous over some of the reported visible spectrophotometric methods with respect to, reproducibility, precision, accuracy and stability of the coloured species. The proposed method is suitable for the determination of phenylephrine in pure form and in nose drop-formulation without excipients interference .

REFERENCES

- Al-Abachi, M. Q. ; Al-Ward, H. S. (2002). Spectrophotometric micro determination of phenylephrine hydrochloride in pharmaceutical preparations via oxidative coupling with 4-aminoantipyrine in the presence of sodium periodate. *National J. Chem.*, **6**, 221-230.
- Amer , M. M. ; Taha , A. M. ; El-Shabouri , S. R. ; Khashaba , P.Y.(1982). Spectrophotometric determination of ephedrine hydrochloride and phenylephrine hydrochloride. *J. Ass. off. Anal. Chem.*, **65**,894-898.
- Amer , S. M.; Abbas, S. S. ; Shehata, M. A. ; Ali, N. M. (2008). Simultaneous determination of phenylephrine hydrochloride, guaifenesine and chlophairamine maleate in cough syrup by gradient liquid chromatography. *J. AOAC, Int.*, **91**(2), 276 -284.
- Auerbach, M. E. (1950). Colorimetric determination of phenylephrine (neo synephrine) in pharmaceutical preparations. *J. Am. Pharm. Assoc.* **50** (2) , 39, 50-52; *Anal. Abstr.*, (1950), **32**, 10d,.
- Beyene, N. W. ; Vanstaden, J. F. (2004). Sequential injection spectrophotometric determination of phenylephrine hydrochloride pharmaceutical preparations, *Talanta* , **63**(3), 599.
- " British Pharmacopeia on CD-ROM ", (2000). 3rd edn., System simulation Ltd, the stationary office, London .
- Deodhar, R. D. ; Mehta, R. C. (1978). Colorimetric estimation of phenylephrine hydrochloride in pharmaceutical formulation. *Indian J. Pharm. Sci.* , **40**(5), 167-169; *Anal. Abstr.*, (1979), **37** , 1E56 .
- Doulakas, (1975). Rapid spectrophotometric determination of phenylephrine hydrochloride in eye drops in presence of other drugs. *J. Pharm. Acta Helv.* **50**(3), 66-68 ; *Anal. Abstr.*, (1975), **29**,4E44.
- Fabrizio, F. D. (2006). Spectrophotometric determination of acetaminophen, phenylephrine hydrochloride , codein phosphate and pyrilamine maleate in tablets or powde . *J. Pharm. Sci.* , **57**(4) , 644-645.
- Gala , B. ; Gomez-Hens, A. ; Perez-Bendito , D.(1994). Individual and simultaneous determination of ephedrine hydrochloride and phenylephrine hydrochloride by use kinetic methodology. *J. Anal. Chem.*, **349**(12),824-828.
- Hargis, L.G. (1988). "Analytical Chemistry, Principles and Techniques", Prentice-Hall Inc, New Jersey. pp. 424- 427.
- Hiskey, C. F. ; Levin, N. (1960) . Colorimetric determination of phenylephrine using 4-aminoantipyrine. *J. Pharm . Sci.* , **50**, 393-395.
- Ibrahim, S. A; Alaa , S. A. (2007). Spectrophotometric microdetermination of phenylephrine hydrochloride in pure and in pharmaceutical formulation using haemotxylin, *J. Molecular Liquids*, **130** (1-3) , 84-87.
- Kazemipour, M. ; Ansari, M. (2005). Derivative spectrophotometry for simultaneous analysis of chlorpheniramine maleate, phenylephrine HCl and phenylpropanolamine HCl in ternary mixtures and pharmaceutical dosage forms. *Iranian J. Pharm. Res.*, **3** , 147-153 .
- Kelly, C. ; Auerbach, M. (2006). Ion exchange separation and Colorimetric determination of phenylephrine in pharmaceutical products. *J. Pharm. Sci.* , **50**(6) , 490-493.

- Knochen, M. ; Giglio, (2004). Flow injection determination of phenylephrine hydrochloride in pharmaceutical dosage forms with on-line solid-phase extraction and spectrophotometric detection . *J. , Talanta* , **64**(5),1226-1232.
- Louis , St. (1985). "Drug Facts and Comparisons." , A Division of J.B. Lippinco company, Toronto, 674p.
- Matthew ,W. R. ; Browne , H.C. ; Weber , J. B.(1972). Rapid method for the determination of numerous pharmaceutical preparations. *J. Ass. off. Anal. Chem. , 55*(4), 789-793 ; *Anal. Abstr.*, (1973), **24**, 1088 .
- Marin, A. ; Barbas , C. , (2004). CE versus HPLC for the dissolution test in pharmaceutical formulation containing acetaminophen , phenylephrine and chlorpheniramine. *J. Pham. Biomed . Anal. , 35*(4), 769-777.
- Muszalska , I.; Zajac, M.; Wrobel, G. ; Nogowska, M. (2000). UV/VIS spectrophotometric method for determination of caffeine and phenylephrine hydrochloride in complex pharmaceutical preparation. Validation of method , *Acta-Pol-Pharm. , 57*, 247-252.
- Neil , H.B. ; Gelenn , A.P. (1971). Analysis of phenylephrine and phenylpropanolamine hydrochloride in combination , *J. Pharm. Sci.*, **60** (8),1229-1231 .
- Samir, J. ; Uma, P. (2005). Synthesis and application of novel 4,5,6,7-tetrahydrobenzothiazole based azo disperse dye. *Arkivoc*,141-149.
- Sane, R.T. ; Narkar , V.S. (1980). Colorimetric estimation of phenylephrine hydrochloride, *Indian Drugs . 18*(1), 23-24; *Anal. Abstr.* (1981), **41**,6E60.
- Senyuva , H. ; Ozden, T. (2002). Simultaneous high-performance liquid chromatographic determination of paracetamol , phenylephrine hydrochloride and chlophairamine maleate in pharmaceutical dosage forms, *J. Chromatogr. Sci.*, **40** (2) , 97-100.
- Szekeres , L. ; Harmon , E. ; Gupta , S. K. (1973). Spectrophotometric determination of phenylephrine hydrochloride, *Microchem. J.*, **18** ,583-588.
- Tammilehto, S. ; Farmaseuttinen Aikak, (1975).Determination of ephedrine, metaoxedrine [phenylephrine] and isoprenaline in pharmaceutical preparations as dinitrophenyl derivatives, *Farmaseuttinen Aikak , 84*(2), 53-59; *Anal. Abstr.*(1976), **31**, 3E33.
- Tatsuzawa , M. ; Shimoda, M. (1968). Spectrophotometric determination of phenylephrine hydrochloride in pharmaceutical preparations. *Bunseki Kagaku*, **17**(5), 551-555; *Chem. Abstr.* (1969), **69**,46091h .
- Yahia, M. D. ; Laila, N. G. (1976). Colorimetric determination of phenylephrine hydrochloride in pharmaceutical preparation. *Analyst* . **101** ,717-719.
- Yolanda , F.M. ; Luis, L. Z. ; Jose, M.C. (2001). Determination of phenylephrine hydrochloride by flow injection analysis with chemiluminescence detection , *J. AOAC Int. , 84*(1),13-18.