Scientia Pharmaceutica (Sci. Pharm.) **71**, 179–194 (2003)

Utility of Certain π-Acceptors for the Spectrophotometric Determination of Hydralazine Hydrochloride

El-Hawary W F*[1], Shoukry A F[1], Talat A[2].

[1]Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt.
[2]Raw materials control Department, Egyptian organization for Biological products & Vaccines, Giza, Egypt

Abstract

The interaction of hydralazine hydrochloride with picric acid (I), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (II), 2,4-dinitrobenzoic acid (III), bromanil (IV) and chloranil (V) was found to be useful for its spectrophotometric determination. The determination was carried out at 520, 510, 550, 540 and 535 nm for the reaction with (I), (II), (III), (IV) and (V), respectively. The effect of several variables on the colouring process was studied. The proposed methods have been applied successfully for the determination of hydralazine hydrochloride in pure samples and in pharmaceutical preparations with good accuracy and precision. The results were compared to those obtained by the official and pharmacopoeial methods. The linear ranges for obedience of Beer's law are up to 118, 54, 639, 27.5 and 78.6 mg/l, Ringbom ranges are 13-100, 7.6-44.7, 77.4-445.0, 5.8-25.8 and 22.5-63.1 μg/ml, Detection limits are 4.8, 2.2, 18.4, 1.2 and 5.9 mg/l, and RSD 0.042, 0.037, 0.043, 0.013 and 0.073 for reactions of hydralazine hydrochloride with I, II, III, IV and V, respectively.

Keywords

Hydralazine hydrochloride, Spectrophotometric determination, π-Acceptors.

Introduction

Hydralazine hydrochloride ($C_8H_8N_4.HCl$) [1-hydrazinophthalazine hydrochloride] is an important compound, widely used as antihypertensive drug. It has been used alone or in combination with other compounds such as Serpasil, Esedrex and Hydrochlorothiazide. The CAS Registry No. is [304-20-1] for the hydrochloride salt.

Several analytical procedures have been adapted for the assay of hydralazine hydrochloride. They included titrimetry [1, 2, 3], visible spectrophotometry [4, 5, 6, 7, 8], ultraviolet spectrophotometry [9, 10], fluorometry [11, 12], HPLC [13, 14], atomic emission spectrophotometry [15], potentiometry [16, 17], polarography [18], paper chromatography [19], gas chromatography [20] and thin layer chromatography [21]. The official method [2, 3] involves the utility of hydralazine as a titrant in iodometric titration. The present study describes the utility of picric acid (I) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (II), 2,4-dinitrobenzoic acid (III), bromanil [2,3,5,6-tetrabromo-1,4-benzoquinone] (IV) and chloranil [2,3,5,6,-tetrachloro-1,4-benzoquinone] (V), as reagents for the spectrophotometric determination of hydralazine hydrochloride in pure samples and in its pharmaceutical preparations.

Experimental

Apparatus

Perkin . Elmer . Spectrophotometer model Lambda 1, Hanna instrument coductometer model HI8819N and Hanna pH meter model HI3313N were used for measuring absorbance, conductance and pH values, respectively.

Materials

Hydralazine hydrochloride [1-hydrazinopthalazine hydrochloride] was obtained from Sigma, U.S.A, and its purity was determined by the U.S. pharmacopoeia XX method [2] or British pharmcopoeia 2000 [3]. The pharmaceutical preparation (Ser-Ap-Es tablets) was purchased from the local market (Swiss Pharma Co., Egypt). All

reagents were of analytically pure grade. They include sodium hydroxide, citric acid, boric acid, potassium dihydrogen phosphate, barbitone, ethyl alcohol (99%), picric acid, DDQ, 2,4-dinitrobenzoic acid, bromanil and chloranil.

Stock solutions

Hydralazine hydrochloride, 4×10^{-2} M solution was prepared and standardized [2, 3], further dilution were made to 3×10^{-3} M for the reaction with (I), (II), (IV) and (V) and to 9×10^{-3} M for reaction with (III). Aqueous solutions of (I) and (II) were prepared at concentration of $3x10^{-3}$ M, and ethanolic solutions were prepared at concentrations of $9x10^{-3}$ M (III), 2×10^{-3} M (IV) and 3×10^{-3} M (V). The pH of the medium was adjusted using Universal buffer [22] of pH 9 and 10, or concentrated solution (3-5 M) of sodium hydroxide.

Procedure

To solution containing 5.8-25.8, 22.5-63.1, 13.0-100.0, 7.6-44.7, 77.4-445.0 μg/ml of hydralazine hydrochloride, a known excess (5 folds) of (I), (II), (III), (IV) or (V) was added at room temperature. The volume was then completed to 10 ml with sodium hydroxide to give 4.2 M in case of (I), with Universal buffer pH 10 in case of (II) and IV, sodium hydroxide to give 0.02 M in case of (III) and Universal buffer of pH 9 in case of (V). The complexes were formed after 20 and 15 minutes for (I) and (II), and after10 minutes for (III), (IV) and (V), and remained stable for 40, 60, 180, 20 and 80 minutes for (I), (II), (III), (IV) and (V), respectively. The absorbances were then measured at 520, 510, 550, 540 and 535 nm, for the five reagents, respectively.

Application to pharmaceutical preparations

The developed procedure was applied for the determination of hydralazine hydrochloride in some dosage forms without prior separation. Twenty tablets of (Ser-Ap–Es) were weighed accurately and powdered in a mortar. An amount

corresponding to 100 mg of hydralazine hydrochloride was transferred to 100 ml measuring flask containing 70 ml distilled water. The suspension was shaked with a mechanical shaker for 30 minutes, followed by treating for 1 minute in a bath subjected to the action of ultrasonic waves then diluted to the mark with distilled water. The solution was filtered and the first 25 ml of the filtrate were rejected. Then 25 ml portion of the filtrate was collected into 50 ml measuring flask, the volume was completed to the mark with distilled water. To solution containing 5.8-25.8, 22.5-63.1, 13.0-100.0, 7.6-44.7, 77.4-445.0 μg/ml of hydralazine hydrochloride, a know excess (5 folds) of (I), (II), (III), (IV) or (V) was added at the optimum conditions of the reactions and the absorbances were measured at the specified wavelengths. The concentrations of the drug were obtained from the calibration curves of hydralazine hydrochloride, and the recoveries applying the new method were calculated.

Results and Discussion

Determination of hydralazine hydrochloride

Picric acid (I), DDQ (II) and 2,4-dinitrobenzoic acid (III) have many applications for the analysis of different electron donor compounds [23,24]. Electron donation from the hydralazine ring occurs due to the presence of two nitrogen atoms in the ring, thus increasing the charge density on it. So, these reactions based on the utility of the π-acceptor reagents (I, II and III) to form a charge transfer complex with the electron donor hydralazine ring.

Bromanil (IV) and chloranil (V) are easily and rapidly reduced [25] by hydralazine hydrochloride to 1,4-tetrabromohydroquinone (TBHQ) and 1,4-tetrachlorohydroquinone (TCHQ), respectively, since hydralazine hydrochloride has strong reducing properties [1, 2, 3].

Hydralazine hydrochloride was found to yield intensely red, red and blue coloured products in case of reaction with (I), (II) and (III), respectively. These complexes exhibit maximum absorbances at 520, 510 and 550 nm, respectively, most probably due to charge transfer complexation between hydralazine as n-donor

and these reagents as π-acceptors, with the subsequent formation of the highly coloured anion radical. Figure 1 shows the absorption spectra of the coloured product of hydralazine hydrochloride with (I), (II), (III), against a reagent blank.

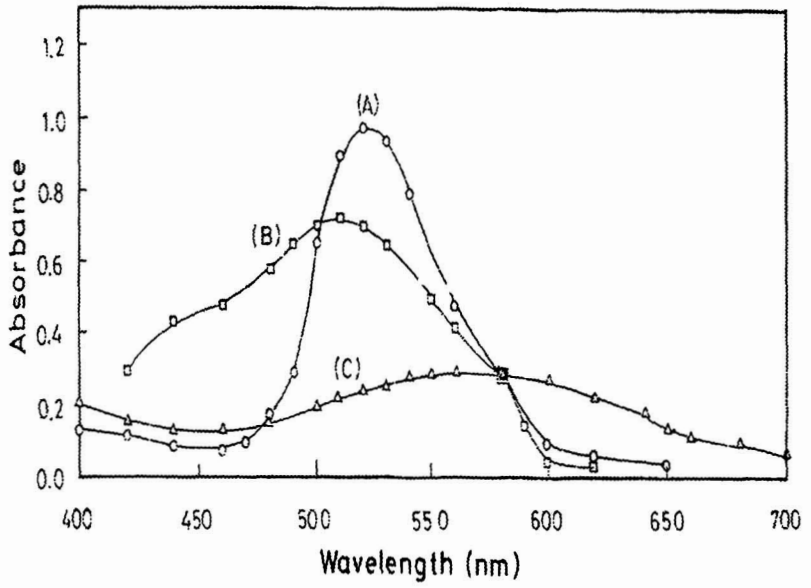

Fig. 1. Absorption spectra of hydralazine reaction products with:
(A) Picric acid (B) DDQ) (C) 2,4-dinitrobenzoic acid
Final concentrations of hydralazine are $3X10^{-3}$ M (A & B) and $9X\ 10^{-3}$ M (C).

In case of (IV) and (V), violet colour was produced in Universal buffers of pH 10 and 9, respectively. This colour disappears by adding hydralazine hydrochloride as a result of reduction to hydroquinones, thus leading to product having less molar absorptivity than the parent reagent (IV or V). The decrease in absorbances of (IV) and (V) at 540 and 535 nm, respectively, was used for the determination of hydralazine hydrochloride. Figure 2 shows the absorption spectra of the reaction products of hydralazine hydrochloride with (IV) and (V). The figure shows that the reduction products with (IV) or (V) have no absorbances at the wavelength of

maximum absorption of the reagents. The colours disappear by adding hydralazine hydrochloride as a result of reduction to (TBHQ) or (TCHQ).

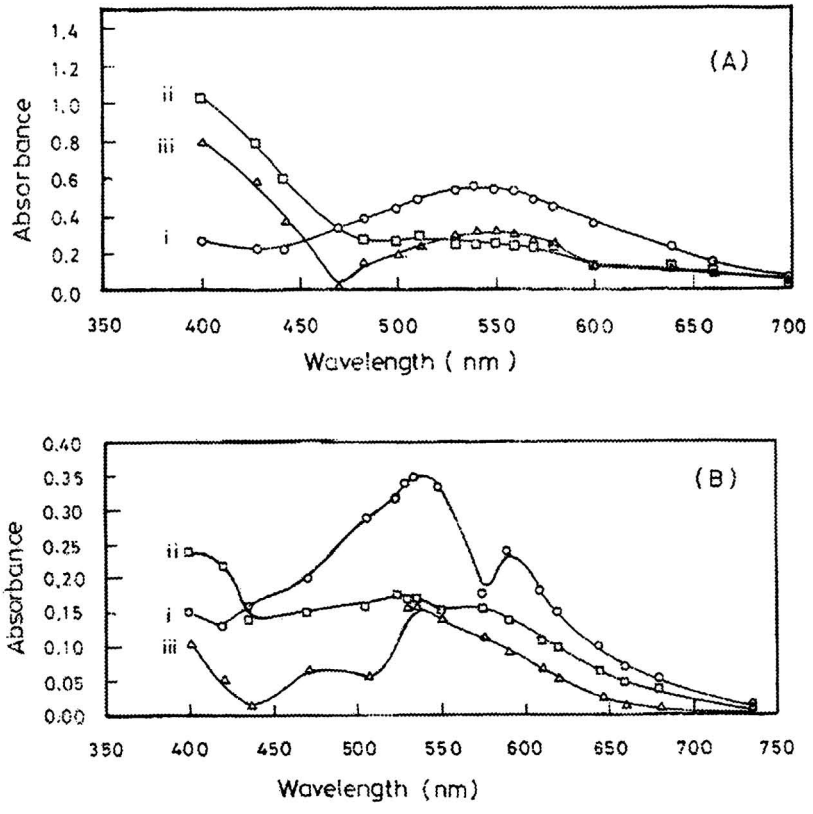

Fig. 2. Absorption spectra of hydralazine reaction products with:
(A) Bromanil and (B) Chloranil
Concentration of hydralazine = $3X10^{-3}$ M in both cases.

Application of molar ratio [26], Job's continuous variation [26] and conductometric titration [22] methods, indicates the formation of 1:1, 2:1 and 1:1 in

case of (I), 1:1, 1:1 and 1:1 in case of (II) and 1:2 & 1:3, 1:2 & 1:3 and 1:3 in case of (III) (hydralazine: reagent) complexes, respectively.

The formation of these complexes was utilized for the spectrophotometric determination of hydralazine hydrochloride, by measuring the absorbances of the formed complexes in case of (I), (II) and (III) at 520, 510 and 550 nm, respectively, and measuring the decrease in absorbances in case of (IV) and (V) at 540 and 535 nm, respectively. The results shown in Table 1 reveal that up to 118, 54, 639, 27.5 and 78.6 mg/l of hydralazine hydrochloride can be determined using (I), (II), (III), (IV) and (V), respectively. The detection limits (DL) are 4.8, 2.2, 18.4, 1.2 and 5.9 mg/l, respectively.

Beer's law range, molar absorptivity, specific absorptivity, Sandell's sensitivity, Ringbom range, and standard deviation of the calibration graph were summarized in Table 1.

Reagent	ε	a	i	r	S	$s\times10^5$	Conc. mg/l	Ringbom range μg/ml
I	2.01×10^3	10.20	0.18	0.94	0.142	9.80	4.8 - 118.0	13.0- 100.0
II	3.90×10^3	19.83	0.02	0.99	0.556	5.08	2.2 - 54.0	7.6- 44.7
III	6.10×10^2	3.12	0.03	0.99	0.110	32.05	18.4 - 639.0	77.4- 445.0
IV	2.23×10^3	11.34	9.4×10^{-3}	0.99	7.8×10^{-3}	8.44	1.2 - 27.5	5.8- 25.8
V	1.14×10^3	5.80	0.03	0.96	5.8×10^{-2}	17.20	5.9 - 78.6	22.5- 63.1

I, II, III, IV and V are picric acid, DDQ, 2,4-dinitrobenzoic acid, bromanil and chloranil, respectively; ε: Molar absorptivity; a: Specific absorptivity; i: Intercept of calibration curve; r: Correlation coefficient of the calibration curve; S: Standard deviation of the calibration curve; s: Sandell sensitivity; * (Range for obedience of Beer's law).

Tab. 1. Analytical parameters of hydralazine complexes.

The applicability of the proposed methods was tested for the determination of hydralazine hydrochloride in pharmaceutical preparations, the results are cited in Table 2. It was found that the proposed methods can be applied successfully for the determination of hydralazine hydrochloride both in pure form and in pharmaceutical preparations as shown by the good recoveries obtained (98.70-101.20%). The precision of the method was tested by calculating the relative standard deviations; the results are shown in Table 2. The low value of relative standard deviation is taken as an evidence for the high precision of the present method.

The mean values obtained by the proposed methods were compared with each other using t-test. Also, their variances were compared with those of the official method [2] using F-test. The obtained values of t and F (F-values ranges from 1.2 to 3.6, t-values from 0.16 to 1) are less than the tabulated values at 99% confidence level. This means that there is no significant difference in accuracy of the proposed methods. Also, the proposed methods are of comparable precision with the official ones at 99% confidence level as shown by the low values of t and F (Tables 2 & 3).

The effect of interference of different cations and anions on the absorbances of the formed complex was studied and it was found that in case of hydralazine complexes with bromanil, chloranil, picric acid, DDQ and 2,4-dinitrobenzoic acid, up to 20 folds of Na^+, Ba^{2+}, Mg^{2+}, Pb^{2+}, Cl^-, NO_2^-, $S_2O_3^{2-}$, SO_4^{2-}, oxalate, citrate, tartrate, salicylate, acetate, nitroprusside, gluconate, pyroborate, hydrogen tartrate, sucrose, lactose, dextrose, glutamine, glycine L-aspragine and EDTA do not interfere. On the other hand, Ca^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}, CN^-, SCN^-, MnO_4^-, $Cr_2O_7^{2-}$, I^-, Br^-, and metavanadate interfere. In case of complexes of picric acid, DDQ and 2,4-dinitrobenzoic acid, Ba^{2+} interferes.

The pharmacopoeial methods [2, 3] for determination hydralazine hydrochloride in the raw material, tablets, and injection depends on titration with potassium iodate in strongly acidic solution, using chloroform to detect the presence of iodine in case of USP method or calomel reference electrode and a platinum indicator electrode in case of BP method. The minimum quantities that

Reagent	Pure solution						Tablet solution					
	mg taken	mg found	% R	S	RSD	F Value	mg taken	mg found	% R	S	% RSD	F-Value*
I	0.25	0.247	99				0.20	0.19	98.6			
	0.40	0.39	99.5				0.40	0.40	100			
	0.70	0.70	100	0.030	0.042	3.63	0.60	0.60	100	0.025	0.03	3
	0.90	0.92	100.5				0.80	0.80	99.99			
	1.10	1.09	99.6				1.00	0.98	98			
							1.20	1.19	99			
II	0.15	0.149	99				0.10	0.090	98			
	0.25	0.25	100				0.20	0.19	99			
	0.35	0.35	100	0.026	0.037	1.52	0.30	0.30	100			
	0.45	0.45	100				0.40	0.40	101	0.030	0.04	1.2
	0.50	0.49	99				0.50	0.50	100			
III	1.00	1.00	100				1.20	1.18	98			
	1.50	1.52	100				2.40	2.40	100			
	2.50	2.53	101	0.035	0.043	1.98	3.00	3.04	101	0.052	0.05	1.9
	3.50	3.50	99.8				4.00	3.95	99			
	4.00	4.00	100				5.00	4.92	99			
	5.50	5.50	99.8									
IV	0.04	0.04	99				0.078	0.078	100			
	0.06	0.06	100				0.11	0.11	99.8			
	0.16	0.16	100	0.002	0.013	2.6	0.16	0.16	100	0.001	9.15×10^{-3}	2.1
	0.18	0.186	100.4				0.18	0.18	100			
	0.23	0.236	101.2				0.20	0.20	100.7			
V	0.10	0.10	100				0.20	0.198	99			
	0.25	0.25	100				0.30	0.30	99	0.017	0.028	2.04
	0.30	0.30	99.5	0.021	0.073	1.88	0.40	0.40	99.5			
	0.45	0.45	100				0.50	0.50	100			
	0.60	0.59	99				0.60	0.60	100			
							0.65	0.65	100			

I, II, III, IV and V: See footnote of table 1; R: Recovery; S: Standard deviation; RSD: Relative standard deviation; *: F- value with respect to official method, (Tabulated F-value at 99%, $\nu_1=6$ (official method) and $\nu_2=5$ (new method) is 4.95) where ν is the degree of freedom

Tab.2. Determination of hydralazine hydrochloride in pure solutions and tablets

Reagent	I	II	III	IV	V	Official method [2,3]
I	----------	0.130	0.163	0.952	0.926	0.345
II	----------	---------	0.388	0.515	1.005	0.417
III	----------	---------	----------	0.410	0.877	0.682
IV	----------	---------	----------	-------	0.596	0.727
V	----------	---------	----------	-------	----------	0.596

I, II, III, IV and V: See footnote of table 1.
(Tabulated t-value at 12 degree of freedom and at 99% confidence level is 3.06).

Tab. 3. Comparison between the different methods used for the determination of hydralazine hydrochloride using t-test.

determined by these methods are 150 mg (USP) or 80 mg (BP), as well as the high volatility of iodine. The proposed methods are used for determination of much lower concentration (5.8 μg/ml) as well as it has a high reproducibility.

Spectrophotometric methods have been used for determination of hydralazine hydrochloride. One method [4] is based on reaction of hydralazine with chloranilic acid dissolved in acetonitril to form a coloured product. The absorbance of this product is measured against a reagent blank at 522 nm, after extraction from tablet in alkaline medium by chloroform, then evaporate till dryness and the residue was dissolved in acetonitril. Recovery of this method is 99-102% and Beer's law was valid from 0.04-0.16 mg/ml. This method needs many extraction steps, which decrease the reproducibility of the method and a long time for determination. Another spectrophotometric method [5] based on diazotization and coupling of hydralazine hydrochloride with 2-hydroxy-1-naphthaldehyde forming hydrazone with absorption maximum at 408 nm. Extraction from tablets, and extraction of the hydrazone product must be done before measurements. The calibration graph was rectilinear from 0.4 to 6 mg/ml and the coefficient of variation was 1.69%. Another spectrophotometric method [6] depends on the reaction between hydralazine and DDQ and measuring the absorbance in acetonitrile at 460 nm. Beer's law is obeyed in the range 32-160 μg/ml and ε is equal to 1.07×10^4. This method needs

extraction with $CHCl_3$ then evaporation and dissolution of the residue in acetonitrile prior to determination, which affect the reproducibility of the method, as well as it is time consuming. While the proposed method is applied to the determination of hydralazine in some dosage forms without prior separation, recovery experiments were carried for each drug in its respective formulation without extraction, time saving, simple and the reagents are commonly used.

Atomic emission technique [15] depends on mixing the powdered tablets with 10 mM ammonium reineckate, after 15 min the solution was filtered, and the equilibrium metal ion concentration in the filtrate was determined by direct coupled plasma-AES. The calibration graph constructed using Cr(III) in 1 M HNO_3 with measurements at 267.71 nm, was linear from 0.1-1000 μg/ml and could be determined in the range 0.19-1.96 mg/25ml. The recoveries are 100-100.3% and RSD of 0.26-1.64%. This method needs expensive technique (Which is not available in all laboratories). Also, it includes several steps and need a very long time for determination and certain specifications and precautions. While the proposed methods do not need any abnormal precaution, time saving, one step reaction and can be run in any laboratory.

All chromatographic techniques [13, 14, 19, 20] are very expensive because they need a certain column for each technique. Also, high purity chemicals are required, and they are time consuming, however they are very accurate and have low detection limits.

Determination of stability constant

In case of the complexes formed between hydralazine hydrochloride and (I), (II) or (III), the overall formation constants (Table 4) were calculated using Harvey and Manning method [28]. The stability constants, β_n, of the formed complexes were calculated using MR and CV methods by the aid of the following equation:

$$\beta_n = \frac{A / A_m}{(1 - A / A_m)^{n+1} C_D^{\,n} n^2}$$

Where, A is the absorbance at the drug concentration C_D; .A_m is the absorbance at full colour development; n is the stoichiometric ratio of the complex; and C_D is the concentration of drug.

Complex of	Method	Mol. ratio	K	β_n
I	MRM	1:1	1.05×10^5	1.05×10^5
II	MRM	1:1	9.41×10^4	9.41×10^4
	CVM	1:1	7.90×10^4	7.90×10^4
III	MRM	1:2, 1:3.	2.26×10^9, 4.80×10^{11}	1.08×10^{21}
	CVM	1:2, 1:3.	1.97×10^9, 6.35×10^{13}	1.25×10^{23}

I, II, III, IV and V: See footnote of table 1; MRM: Molar ratio method; CVM: Continuous variation method; K: Formation constant; β_n: Overall formation constant

Tab. 4. Stability constants of hydralazine hydrochloride complexes.

It was found that the sequence of increasing stability of the complexes is II < I < III. So the compound III (2,4-dinitrobenzoic acid) is the most stable and it is the best one which can be used for determination of hydralazine hydrochloride.

Conclusion

The proposed procedures are simple, time saving, inexpensive and more sensitive than the official methods as shown by the very low detection limits (1.2-18.4 mg/l). Comparing these procedures with the official ones using F-test reveal that there is no significant difference in accuracy between them as shown by the low F-value (1.2-3.6). The developed procedures are applied to the determination of hydralazine in some dosage forms without prior separation. Recovery experiments were carried out for each drug in its respective formulation.

The excellent recoveries obtained (98.70-101.20%) indicate the absence of interference from frequently encountered excipients or additives.

References

[1] Sandri G C.
Volumetric determination and microchemical behaviour of monohydrazinophthalazine and dihydrazinophthalazine
Boll. Chim. Farm. 1957; 96: 431-6, C. A. 1958; 52: 4109g.

[2] United States Pharmacopoeia XX
Official monograph/Hydralazine.
Mack Publishing Co., Easton, Pa. 1980; 377.

[3] British pharmacopoeia.
Monographs: Medical and pharmaceutical substances/Hydralazine hydrochloride.
Her Majesty, Stationery office, England, 3rd ed, 2000; 811.

[4] Mahrous M S, Issa A S, Abdel Salam M A, Soliman N.
Spectrophotometric determination of some cardiovascular drugs using p-chloranilic acid.
Anal. Lett. 1986; 19: 901-14.

[5] Mari-Buigues J, Manes-Vinuesa J, Gracia-Domenech R, Pous- Miralles G.
Spectrophotometric determination of hydralazine with 2-hydroxy-1-naphthaldehyde in pharmaceuticals.
J. Pharm. Sci. 1991; 80: 690-2.

[6] Issa A S, Mahrous M S, Abdel Salam M A, Soliman N.
The use of 2,3-dichloro-5,6-dicyano-p-benzoquinone for the spectrophotometric determination of some cardiovascular drugs
Talanta. 1987; 34: 670-2.

[7] Ruggieri R.
Hydrazinophthalazine I. determination
Farmaco (pavia) ed. Part. 1956; 11: 571-6, C. A. 1959; 53: 14421a.

[8] Luk'Yanchikova G I.
Photocolourimetric determination of phtalazol, sulfodiamine, anesthesin, and apressine.
Med. Prom. SSSR. 1962; 16: 46-8; C. A. 1963; 58: 5454h.

[9] Barary M H, Elsayed M A, Mohamed S M.
Spectrophotometric determination of hydralazine hydrochloride, oxprenolol hydrochloride and chlorthalidone in combination and for oxprenolol hydrochloride as single component dosage form.
Drug Dev. Ind. Pharm. 1990; 16: 1539-54.

[10] Mopper B.
Ultra-violet spectrophotometric determination of hydralazine hydrochloride in tablets following derivatization with nitrite.
J. Assoc. Off Anal. Chem. 1987; 70: 42-46.

[11] Barary M H.
Spectrofluorimetric determination of hydralazine hydrochloride using 1-Dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride).
J. Pharm. Belg. 1991; 46: 247-51.

[12] Danielson N D, Bartolo R G.
Determination of hydralazine in tablets by fluorescence.
Anal. Lett. 1983; 16: 343-54.

[13] Kim M, Stewart J T.
HPLC post-column derivatization of aromatic amines using N-methyl-9-chloroacridinium triflate.
Mikrochim. Acta. 1990; 3: 221-32.

[14] United States Pharmacopoeia XXIV, NF. XVIV,
Official monograph/Hydralazine
Asian ed., US Pharmacopeial Convention inc., MD. 2000; 818.

[15] Issa Y M, Shoukry A F, Ibrahim H, Mohamed S K.
Atomic-emission spectrometric determination of antazoline, hydralazine, amiloride, thiamine and quinine based on formation of ion associates with ammonium reineckate.
Anal. Lett. 1994; 27: 731-42.

[16] Sakharov A A.
Potentiometric determination of apressin (hydralazine).
Khim. Farm. Zh (Russ). 1989; 23: 1395-96, C. A. 1990; 112: 62767v.

[17] Badawy S S, Shoukry A F, Rizk M S, Omar M M.
Hydralazine-selective PVC membrane electrode based on hydralazinium tetraphenylborate.
Talanta. 1988; 35: 487-89.

[18] Modras Z.
Polarographic method for the evaluation of the stability of aqueous solutions of 1-hydrazinophthalazine hydrochloride (Apresoline) and 1,4-dihdrazine-pthalazine sulfate (Nepresol).
Chem. Anal. (Warsaw). 1973; 17: 1349-53; C. A. 1973; 78: 128457g.

[19] Zimmer H, Glaser R, Kokosa J, Garteiz D A, Hess E V, Litwin A.
3-Hydroxymethyl-5-triazolo[3,4-a] phthalazine, a novel urinary hydralazine metabolite in man.
J. Med. Chem. 1975; 18: 1031-3, C. A. 1975; 83: 188169c.

[20] Smith K M, Johnson R N, Kho B T.
Determination of hydralazine by gas chromatography.
J. Chromatogr. 1977; 137: 431-7, C. A. 1977; 87: 73417x.

[21] Zak S B, Bartlett M F, Wagner W E, Gillerna T G, Lukas G.
Disposition of hydralazine man and a specific method for its determination in biological fluids.
J. Pharm. Sci. 1974; 63: 225-9, C. A. 1974; 80: 140963t.

[22] Vogel A I.
Vogel's Text Book of Quantitative Chemical Analysis", ELBS, Longman, London, 5th ed., 1989: 519, 831.

[23] Badawy S S, Shoukry A F, Issa Y M.
Molecular compounds of 1-hydrazinophthalazine hydrochloride with aromatic di- and tri-nitro compounds. Preparation and infrared spectroscopic investigation
Egypt. J. Chem. 1991; 34: 41- 49.

[24] Saleh G A.
Charge-transfer complexes of barbiturates and phenytoin.
Talanta. 1998; 46: 111-21.

[25] Cybulski D, Male K B, Scharer J M, Moo-Young M, Luong J H T.
Substrate recycling scheme for tetrachloro-p-benzoquinone using bilirubin oxidase and NADH: application for pentachlorophenol assay.
Environmental Science and Technology. 1999; 33: 796-800.

[26] Skoog D A, West D M.
Fundamentals of Analytical Chemistry, Holt, Rinehardt and Winston Inc., USA, 2nd ed., 1969: 679, 682.

[27] Harvey E, Manning D L.
Spectrophotometric methods of establishing empirical formulas of coloured complexes in solution.
J. Am. Chem. Soc. 1950; 72: 4488-4493.

[28] Ringbom A.
Accuracy of colourimetric determinations.
Z. Anal Chem. 1939; 115: 332-43.

Received March 12th, 2003
Accepted June 2nd, 2003