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FORMAZAN-INDUCED COLORIMETRIC ANALYSIS OF DIHYDRALAZINE SULFATE

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Abstract-A colorimetric assay procedure was developed for the quantitative analysis of dihydralazine sulfate. The method is based on the interaction of 2,3,5-triphenyltetrazolium chloride with dihydralazine sulfate, in presence of potassium hydroxide in absolute ethanol, to afford a highly colored and stable formazan. This could be spectrophotometrically quantified at 485 nm, with an ideal adherence to Beer's law for solutions containing 2.0-22.0 mcg dihydralazine sulfate/ml. The presented color reaction is selective;no interference could be displayed by reserpine or hydrochlorothiazide. This permitted the application of the procedure to the satisfactory analysis of commercial dihydralazine formulations without prior separation from these drugs.

I N T R O D U C T I O N

Of the several, clinically adopted antihypertensive agents, dihydralazine (1,4-dihydrazinophthalazine) is distinguished with a unique ability to improve cerebral and renal blood flows (1). For an integrated therapeutic effect dihydralazine sulfate is oftenly formulated with reserpine and hydrochlorothiazide (2).

Much interest has been devoted to the quantitative estimation of dihydralazine, which can be achieved by the application of several oxidimetric (3-7) ,complexometric (8,9),polarographic (10),coulometric (11,12) ,

and colorimetric measures. The chromogenic reagents reported involve ammonium molybdate (13),ferric-iron (14), dimethoxydiquinone (15) and 2-methyl-3-nitropyridine-6-carboxaldehyde (16). Such a vast collection of methods would in turn reflex the difficulty encountered to establish an ideal procedure for the analysis of this drug; either because of considerable lack of sensitivity and specificity (3-9,13-14) or due to uncommon availability of the assay reagents (15-16).The same token can be extended to the compendial analysis of dihydralazine (17) since it utilizes a nonselective oxidimetric procedure (15-16).

In the context of the foregoing discussion, an existing need calls upon to develop a more convenient,accurate and specific method for the analysis of dihydralazine, and in particular, for its dosage forms.

It has been observed in this laboratory, that 2,3,5-triphenlytetrazolium chloride (TTC) can be selectively reduced into the corresponding highly colored formazan derivative, when interacted with aromatic diols (18,19). Since dihydralazine is ascribed with a strong reducing affinity (6,13),it seemed worthwhile to investigate its interaction with TTC. The presented contribution offers a simple,senstive and specific colorimetric procedure for the analysis of dihydralazine sulfate, both in its pure form and tablet formulations.

E X P E R I M E N T A L

Instrumentation-A double-beam UV-Visible spectrophotometer[1] with matched pairs of 1-cm glass cells, a pH meter[2] fitted with a sealed calomel and shielded glass electrodes, and an analytical balance[3] were used.

Materials-Pharmaceutical grade dihydralazine sulfate was utilized as the working standard.All other chemicals were analytically pure.As dosage forms, two commercially available dihydralazine tablet formulations[4,5] were analyzed.

Reagents-1-Tetrazolium Solution : dissolve 0.5 g of pure TTC[6] in 100 ml of absolute ethanol. This reagent must be kept in amber-colored glass containers and cooled well when not in use, otherwise it should be freshly prepared every 3 days.

2-Potassium Hydroxide Solution : prepare 0.025%(w/v) carbonate-free solution in absolute ethanol, using ca. 1 ml of distilled water to aid solubilization. This solution keeps well for not more than 3 days, after which it should be freshly prepared too.

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[1] Spektromom-203,MOM,Hungary.
[2] Radelkis OP-401/2,Hungary.
[3] WA-33,Poland.
[4] Adelphane (CIBA,Switzerland),contains 10.0 mg dihydralazine sulfate and 0.1 mg reserpine per tablet.
[5] Adelphane-Esidrex (CIBA),contains 10.0 mg dihydralazine sulfate, 0.1 mg reserpine and 10.0 mg hydrochlorothiazide per tablet.
[6] Merck (Germany).

Standard Solutions-Dissolve an accurately weighed amount of the well dried dihydralazine sulfate in absolute ethanol so as to obtain a standard concentration of 100 mcg of the sulfate salt/ml.

Assay Samples-Place a single powdered tablet, or its equivalent from a composite of 20 tablets, in a 100-ml volumetric flask, add 50 ml absolute ethanol and allow to stand for 30 minutes with frequent shaking.The mixture is completed to volume with absolute ethanol,mixed well and filtered through a dry filter into a dry flask, the first portions of the filterate being rejected. One ml of the filtered solution is supposed to contain 100 mcg of the claimed dihydralazine sulfate content.

Assay Procedure-Pipet 1.0 ml of the standard or assay sample solution into a 10-ml volumetric flask that contains 5.0 ml of the tetrazolium reagent and 2.0 ml of potassium hydroxide assay solutions,bring to volume with absolute ethanol, mix well and leave at room temperature for 20 minutes,protecting the solution from direct light. Transfer the red-colored solution into a 1-cm glass cell and determine light absorption at 485 nm versus a blank prepared from 1.0 ml absolute ethanol and treated similarly.

RESULTS AND DISCUSSION

Dihydralazine-TTC Interaction- On account of their marked weak oxidizing power, tetrazolium salts are only selectively affected by organic reducing compounds(18-21). An attractive feature of this redox interaction is associated with the formation of highly colored formazans, that

usually exhibit strong light absorption.This has long justified the utility of TTC as a senstive spot reagent for detection of strong reducing agents (20). When extended to pharmaceutical analysis, this interaction still reflexes a such high sensitivity, that enables microdetermination of ascorbic acid and catecholamines (21,18) at a subnanogram concentration level.Since most of the identity tests and assay procedures reported for dihydralazine are based on its strong hydrazine-reducing effect (22), a possible TTC-dihydralazine redox interaction is anticipated.Indeed, this interaction readily afforded at room temperature an intensively red-colored species, the light absorption spectrum of which,with maximum extinction at 485 nm,confirmed its formazan nature (18,21). As for most of formazan-induced colorimetric analyses (21),the rate of TTC-dihydralazine interaction was markedly influenced by the following variables :

1-Reagent Concentration-No formazan development can be monitored when dihydralazine sulfate is interacted with TTC in absence of alkalis. As revealed by Figure 1, repetion of the interaction in millieu of varying amounts of potassium hydroxide brought about different rates of color development,with maximum absorbance for an initial concentration of 0.025 % (w/v) KOH in absolute ethanol[7]

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[7]Relative pH of the assayed solution,that contained 1.0 ml standard dihydralazine, 5.0 ml TTC reagent and 2.0 ml of the alkali solutions approximated 10.7.

Apart from its role in enhancing the reduction of the tetrazolium cation (23), the presence of a strong alkali seems indespensable to generate the free,otherwise nonreducing, dihydralazine base.Though not so influencive as the change in hydrogen ion concentration, variation in the amount of TTC interacted with dihydralazine is shown to affect the rate of formazan production,Figure 2. Maximum color formation was attained by addition of 1.0 ml of the standard dihydralazine solution to 5.0 ml of the TTC reagent,that was already mixed with 2.0 ml of 0.025 % KOH solution. Such a mode of addition also proved more effective in raising color absorbance.

2-Time of Interaction-Keeping all other experimental varialbles constant,maximum color absorption was attained after 20 minutes-interaction period at 25°C.Stability of the formazan formed was manifested for not less than 2 hours, Figure 3.

3-Temperature-Though formazan development was relatively improved by carrying out the TTC-dihydralazine interaction at elevated temperature, no practical use was made of this effect on account of the high sensitivity already revealed by the system at room temperature.

No special efforts were made to suggest the nature of the oxidation product(s) of dihydralazine-TTC interaction. Reduction of TTC into its formazan can,in principle,

involve one or both of the hydrazine moieties of dihydralazine, the oxidation pass-way of which can terminate with the formation of 4-hydroxy-1-hydrazino-phthalazine (13) or 2,3-dihydrophthalazine-1,4-quinone (4).

II-Quantitative Analysis- Under the proposed optimum conditions for color production, the quantity of formazan formed was found constant function of the amount of dihydralazine sulfate interacted. A linear regression analysis of Beer's plot at 485 nm afforded a slope value of 0.045 ($\pm$ 4.45 x 10^{-4}) and revealed an excellent adherence (r= 0.9987) over a concentration range of 2.0-22.0 mcg dihydralazine sulfate/ml of the finally assayed solution. This permitted the development of the investigated color reaction into a sensitive spectrophotometric analysis of dihydralazine on account of the high molar absorptivity observed, $\varepsilon_a = 1.297 \times 10^4$. That the proposed procedure is also precise was evidenced by its application to replicate analysis of standard dihydralazine sulfate solutions, Table I, with a relative standard deviation of 8.167×10^{-3}.

Recovery data as assessed by the formazan method proved to be accurate, with a mean percent recovery of 99.90 (± 1.23), Table II. In comparison, the corresponding recovery value for three samples of dihydralazine sulfate pure form, as analyzed according to the compendial method (17) was 101.21.

A favorable characteristic of the TTC method is its specificity for the estimation of dihydralazine in multicomponent pharmaceutical formulations. Reserpine and hydrochlorothiazide fail to induce formazan formation when interacted under the same experimental conditions as for dihydralazine sulfate. This allowed the direct analysis of differently markted dihydralazine tablets without the necessity for prior separation from reserpine or hydrochlorothiazide, Table III.

The presented colorimetric estimation offers a convenient and selective assay procedure for dihydralazine in its pure form and dosage formulations. The common availability of tetrazolium salts as biological reagents and the considerable ease in color development would recommend the application of the method in routine analysis.

Table I-Replicate Analysis of Dihydralazine Sulfate Standard Solutions,[c]; 20 mcg/ ml

Replication		Absorbance, 485 nm
1		0.898
2		0.902
3		0.910
4		0.889
5		0.901
6		0.907
	mean	0.901
	Standard Deviation	7.36×10^{-3}

Table II-Recovery-Analysis of Dihydralazine Sulfate Standard Solutions.

Sample	Dihydralazine Sulfate		SD,±
	Added,mcg/ml	Found, %[a]	
1	4.0	99.30	0.214
2	8.0	98.49	0.396
3	10.0	100.05	0.319
4	12.0	101.50	0.327
5	16.0	100.91	0.394
6	18.0	99.05	0.314
7	20.0	98.56	0.248
8	22.0	101.37	0.354
	Mean percent recovery	99.90	
	Standard Deviation ±	1.23	
	Relative SD	1.2×10^{-2}	

[a]Average of five determinations.

Table III-Analysis of Dihydralazine Tablet Formulations by the TTC method.

Tablet[a]	Dihydralazine Sulfate, mg/ Unit			
	Claimed	Found	Added	Recovered[b]
A	10.0	9.89	10.0	19.90
B	10.0	10.20	10.0	20.20

[a]For detailed composition, cf Experimental.
[b]Average of three determinations.

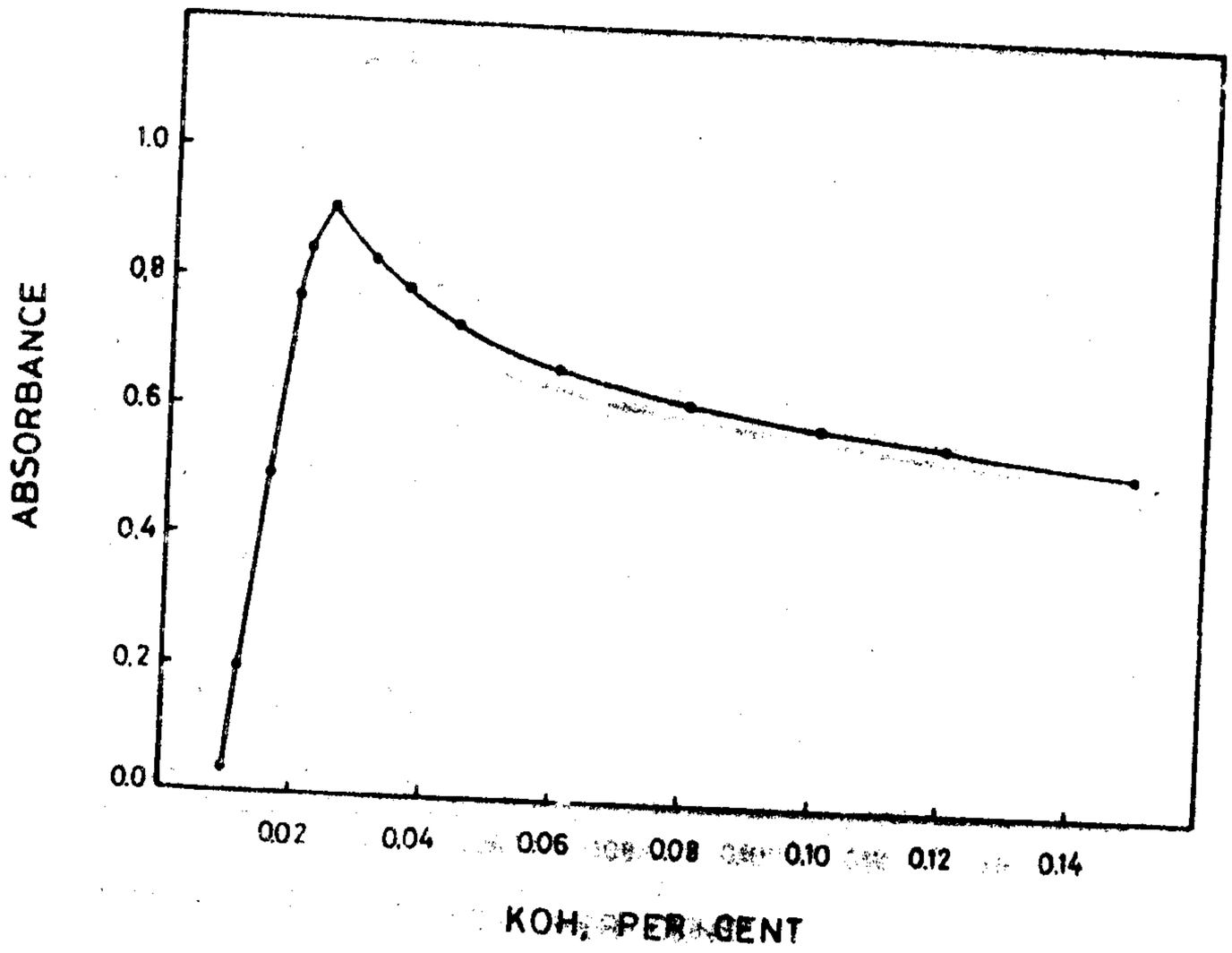

Figure I- Effect of potassium hydroxide on the rate of TTC-dihydralazine interaction at room-temperature, o ; 20 mcg/ ml.

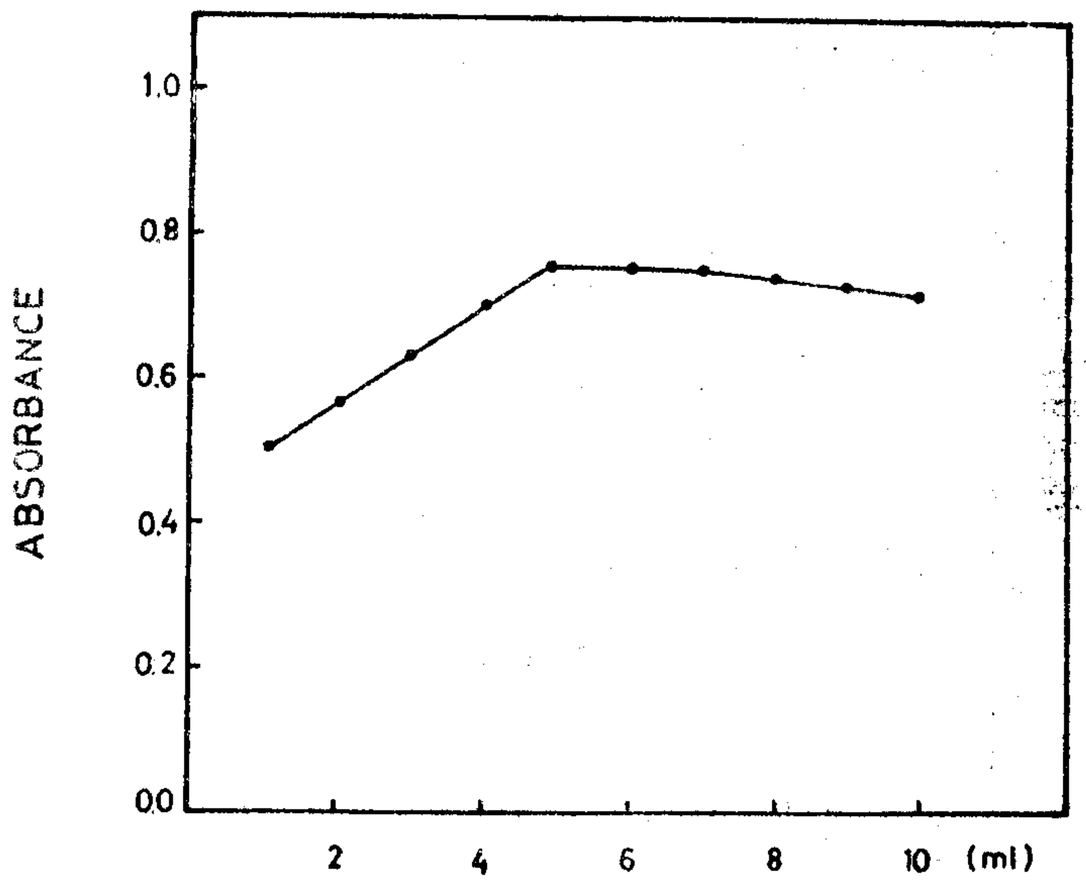

Figure 2- Effect of TTC on the rate of color production at room-temperature, c , I7 mcg dihydralazine sulfate / ml.

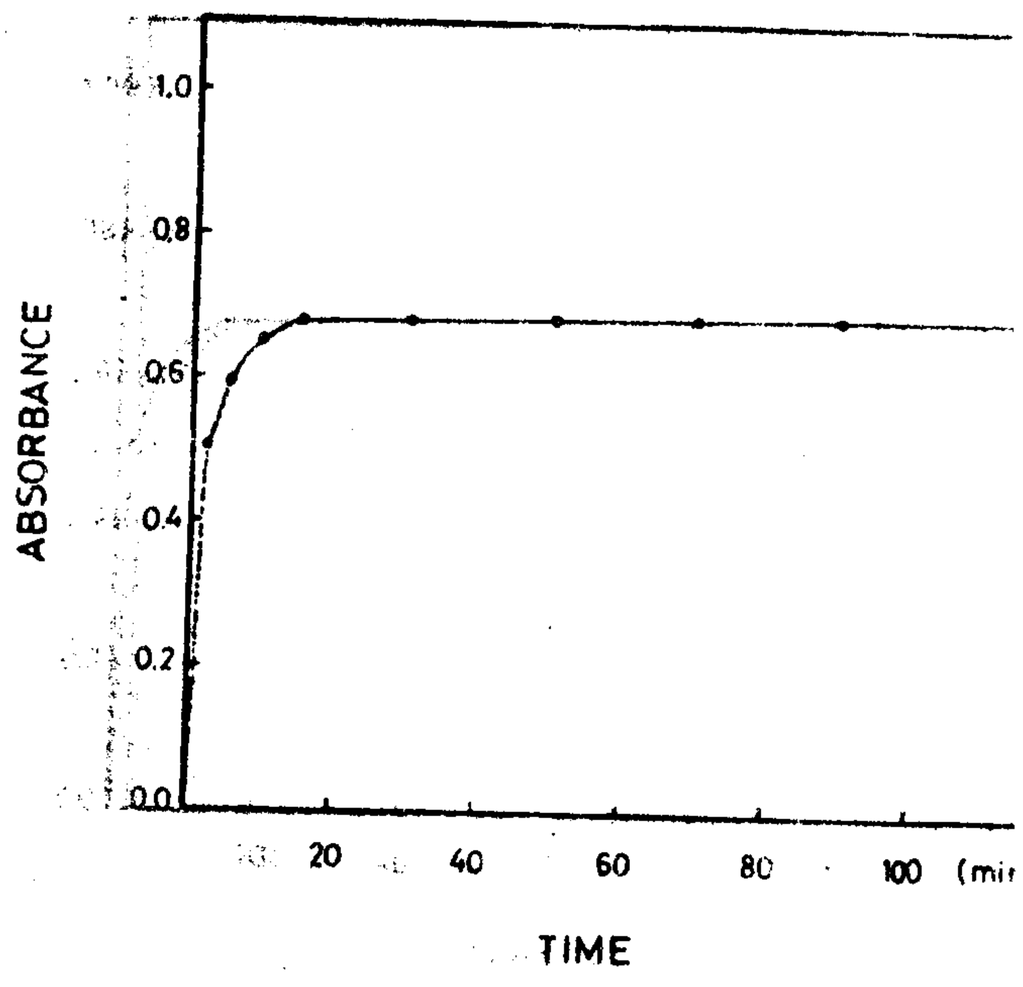

Figure 3- Dihydralazine- TTC interaction a function of time, c , I5 mcg dihydralazine sulfate/ ml.

REFERENCES

(1) S.M.Kirpekar and J.J.Lewis, J.Pharm.Pharmacol., 9, 877 (1957).

(2) "Martindale, The Extra Pharmacopoeia", 26th Ed., The Pharmaceutical Press, London, 1973, p.813.

(3) F.Janick, B.Budešinsky and J.Korbl, Cesk.Farm., 9, 304 (1960).

(4) S.Pinzauti, V. dal Piaz and E.la Porta, J.Pharm.Sci, 63, 1446 (1974).

(5) K.Kalinowski, Farm.Polska, 21, 914 (1965).

(6) R.Soliman and S.A.Belal, Egypt.J.Drug Res., 6, 7 (1974).

(7) N.S.Goryacheva and L.N.Prikhodkina, Farmatsiya, 17, 69 (1968).

(8) I.Grecu and E.Curea, Rev.Roum.Chim, 16, 348 (1965).

(9) ibid., 17, 434 (1966).

(10) Z.Modras, Chem.Anal.(Warsaw), 17, 1349 (1972).

(11) Z.E.Kalinowska, ibid., 9, 83 (1964).

(12) J.Chodkowski and T.Giovanoli., ibid., 17, 603 (1972).

(13) I.Grecu and E.Curea, Rev.Roum.Chim., 16, 389 (1965).

(14) W.Kitzing, Pharmazie, 16, 401 (1961).

(15) E.A.Ibrahim, Master Diss., Assiut, 1976, p.56

(16) A.F.Youssef, S.A.Ibrahim and S.R.Elshabouri, J.Pharm. Sci, 66, 116 (1977).

(17) "The National Formulary", 13th Ed., Mack Publish.Co., Easton, Pa., 1970, p348.

(18) N.M.Omar and N.A.EL-Rabbat, Vth.Congr.Arab Pharm.Union, Kuwait, 1976, p.5

(19) N.M.Omar and N.A.EL-Rabbat, J.Pharm.Sci., 67, in press (1978).

(20) F.Feigel, "Spot Tests in Organic Analysis", Elsevier Publish.Co., Amsterdam, 1966, p.338.

(21) M.H.Hashmi, A.S.Adel, A.V.Viegas and I.A.Ahmed, Mikrochim.Acta ,3,457 (1970).

(22) "Handbuch der Arzneimittel-Analytik", S.Ebel, Verlag Chemie, Weinheim-New York, 1977, p.178-182.

(23) R.Kuhn and D.Jerchel, Ber., 74, 949 (1941).

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تحليل لونى فرمازانى ـ التأثر لكبريتات الهيدرالازين

نوال على محمود الرباط ـ نبيل محمود عمر

قسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة اسيوط

يقدم البحث طريقة طيفية جديدة لتقييم كبريتات الهيدرالازين والمائعة الاستعطاء كمضغات لضغط الدم العالى ـ وذلك بتفاعلها مع الملح الكلوريدى لكاتيون الـ ٢ر٣ر٥ـ ثلاثى الفينيل تترازوليوم فى وسط قلوى مناسب وعند درجات الحرارة العادية ثم قياس الفرمازان الناشئ ـ والذى يتميز بشدة امتصاص الضوء وثباته ـ عند موجه ضوئية طولها ٤٨٥ نم.

وتحت الظروف القياسية للطريقة المذكورة فانه يمكن معايرة ٢ ـ ٢٢ ميكروجرام من كبريتات الهيدرالازين فى صورتها النقية بدقة متناهية .

ولقد ساعدت تخصصية هذا التفاعل واعتماده على الفاعلية الاختزالية المتميزة للمركبات المضوية على تقييم الهيدرالازين فى تراكيبه الصيدلية المختلفة دون تداخل مامن إى من المواد الأخرى المصاحبة له .

وجانب عناصر الدقة والتخصصية العالية فإن الطريقة المستحدثة تتميز أيضا بالبساطة حيث يمكن مباشرة القياس الضوئى بعد عشرين دقيقة من التفاعل تحت ظروف معملية اعتيادية للغاية.

ومما لاشك فيه أن سهولة الحصول على أملاح التترازوليوم ـ والتى تستعمل فاليا ككاشفات للتفاعلات البيولوجية ـ ليضيف الى الطريقة الجديدة عنصراً آخراً من عناصر اليسر فى الأداء .