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SPECTROPHOTOMETRIC DETERMINATION OF PIPERAZINE USING 3,5-DIBROMO-2-METHYL-p-BENZOQUINONE

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ABSTRACT: *A spectrophotometric method is developed for the quantitative determination of piperazine either in bulk drug or in pharmaceutical dosage forms. The method is based on the formation of vinylamine of this drug by interaction with acetaldehyde. The resulting enamine interacts with 3,5-dibromo, 2-methyl-p-benzoquinone (DBMQ) forming an intense blue colored aminovinylquinone which can be estimated spectrophotometrically at* λ_{max} *642 nm. DBMQ is prepared according to a modified oxidation procedure by treatment of 2,4,6 tribromo-3-methylphenol with chromic oxide in a mixture of acetic acid and acetonitrile (9:1). Beer-Lambert's Law was adhered to over the range of 2-8, 2-9, and 2-10* $\mu g.ml^{-1}$ *for piperazine hydrate, tartrate, citrate and phosphate respectively. The optimum reaction conditions and the effect of all variables have been studied. The proposed method is simple, rapid, sensitive, accurate, precise and has been applied successfully for the analysis of some pharmaceutical formulations of piperazine including tablets, effervescent granules and syrup with good recoveries. Results obtained are in good agreement with those obtained by the official methods.*

INTRODUCTION

Piperazine is widely used in Egypt in gout and anthelmintic preparations. The medicinal importance of this drug and its widespread in various pharmaceutical preparations require the availability of a rapid and convenient method for its determination. Piperazine and its salts (phosphate, adipate, and citrate) have been assayed by numerous methods. Among these are gravimetric[1-3] nonaqueous titrimetric[4], chelatometric[5], colorimetric[6-15], GLC [16] and polarographic techniques[17] .

Buckely *et. al.*[18] synthesized some new colored compounds by interaction of vinylamine with chloranil. This reaction is quantitized for the determination of many pharmaceutical secondary amines including piperazine[14,15]. Accordingly in the present work 3,5-dibromo-2-methyl p-benzoquinone (DBMQ) is synthesized according to a modified oxidation procedure[19] and is used for the quantitative estimation of piperazine. The method is based on the reaction of vinylamine of this drug with DBMQ giving the corresponding aminovinyl quinone which can be estimated quantitatively at λ_{max} 642 nm.

EXPERIMENTAL

I- Apparatus:

UVidec-320 Spectrophotometer, Jasco, Tokyo, Japan.

II-Chemicals and reagents:

1- Piperazine hydrate was used as working standard .Its purity was checked and was found to be 99%[4].
2- Piperazine phosphate, citrate and tartrate were obtained from different manufacturers.
3- 3,5-Dibromo-3-methyl p-benzoquinone was synthesized and purified in the laboratories of Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Assiut University. A 0.2% methanolic solution of this reagent was stable for 1 week at ≈ 4°C.
4- Sodium hydroxide solution; alcoholic, about 0.01 N.
5- Acetaldehyde; alcoholic, 5% v/v solution.
6- Solvents used were of analytical grade. (prolabo, France).

III-Pharmaceutical formulations:

1- Parazine tablet (CID); containing 0.3 gm of piperazine monophosphate per tablet.
2- Coliurinal effervescent granules (Misr); containing 0.03 of piperazine citrate per gm of powder.
3- Piperazine citrate syrup (Alex.); containing 0.1 gm anhydrous piperazine citrate per mL.

Preparation of standards:

Weigh, accurately, about 25 mg of piperazine hydrate into a 25 mL measuring flask, dissolve and complete to volume with water. Dilute the solution quantitatively and stepwise to give a series of concentrations suitable for construction of the calibration graph in the specified linear range.

Preparation of samples:

1- *For tablets*: Weigh and finely powder 20 tablets. Transfer an accurate weight of the powder equivalent to 12.5 mg of piperazine, into a 25 mL measuring flask, shake thoroughly with 20 mL water, then complete to volume. Filter and reject the first portion of the filtrate. Dilute the prepared solution quantitatively with water to contain 50 $\mu g.mL^{-1}$ calculated as piperazine base.
2- *For effervescent granules*: Weigh accurately a quantity of effervescent granules equivalent to 50 mg of piperazine. Dissolve in 70 mL water, shake well then complete to 100 mL and filter. Complete as described under *Parazine tablet*," Filter and reject..."
3- *For syrup*: Into a 100 mL measuring flask transfer accurately a measured volume of syrup solution equivalent to 50 mg of piperazine. Complete to volume with water. Transfer 1 mL of the prepared solution into a 10 mL measuring flask and dilute with water to contain 50 $\mu g.mL^{-1}$ calculated as piperazine base.

General procedure:

Pipette 1.0 mL of the prepared solution into a 10 mL measuring flask. Add 0.4 mL of 0.01 N sodium hydroxide, 1 mL of 5% acetaldehyde, 1.2 mL of DBMQ and mix. Heat on a boiling water bath for one minute, cool and complete to volume with ethanol. Measure the absorbance at λ_{max} 642 nm against a reagent blank treated similarly.

RESULTS AND DISCUSSION

Substituted benzoquinones have been previously used for spectrophotometric determination of piperazine and its salts. They function either as π acceptors forming charge transfer complexes[6-9], or as nitrogen nucleophiles which react with vinylamine of piperazine forming colored chromogens[14,15]. In continuation of earlier work, on the analytical uses of these quinones, DBMQ is synthesized in the present study, according to a modified oxidation procedure, and used for estimation of piperazine.

2,4,6-Tribromo-3-methylphenol was quantitatively obtained from the reaction of m-cresol with three equivalents of bromine in acetic acid. Oxidation of halophenols to benzoquinone derivatives has been currently studied using several types of oxidizing agents such as cerric sulphate, cerric ammonium sulphate, manganic sulphate, chromic oxide and periodic acid. With a modified oxidation procedure, treatment of halophenols with an equal molar of chromic oxide in a solvent mixture of acetic acid and acetonitrile (9:1) gave a good yield of 3,5-dibromo-2-methyl-1,4-benzo-quinone (DBMQ).

Direct nucleophilic substitution reaction between DBMQ and piperazine was found to be very slow and weak resulting in a faint red colored product. According to Buckley *et. al.*[18], a way to shift the λ_{max} to longer wavelength and to intensify absorbance of the chromogen formed, is to include an extra conjugation chain using acetaldehyde. The enamine formed react immediately with DBMQ to give the corresponding aminovinylquinone with more intense and longer wavelength. The resulting blue colored product exhibits two maxima, at 322 and at 642 nm (Figure 1).

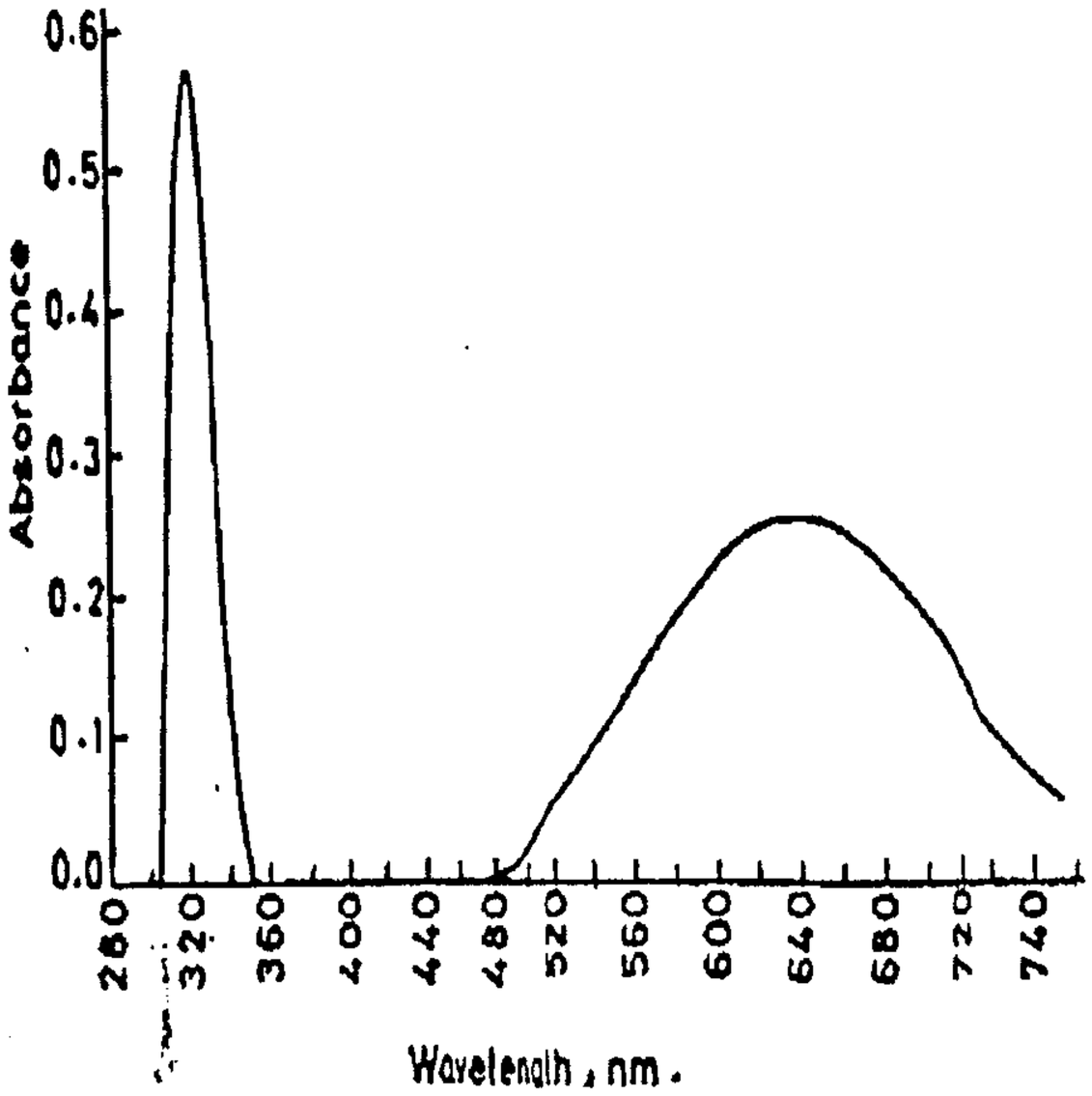

Fig. 1: Absorption spectrum of piperazine :acetaldehyde:DBMQ interaction product.

All variables affecting the reaction product were carefully studied in order to give a stable chromogen of maximum absorption intensity. The presence of acetaldehyde at suitable concentration range is very essential for complete enamine formation. Accordingly, the effect of different volumes of 5% acetaldehyde on the reaction product was studied. Figure 2 indicates that a volume of 1.0 mL is quite enough.

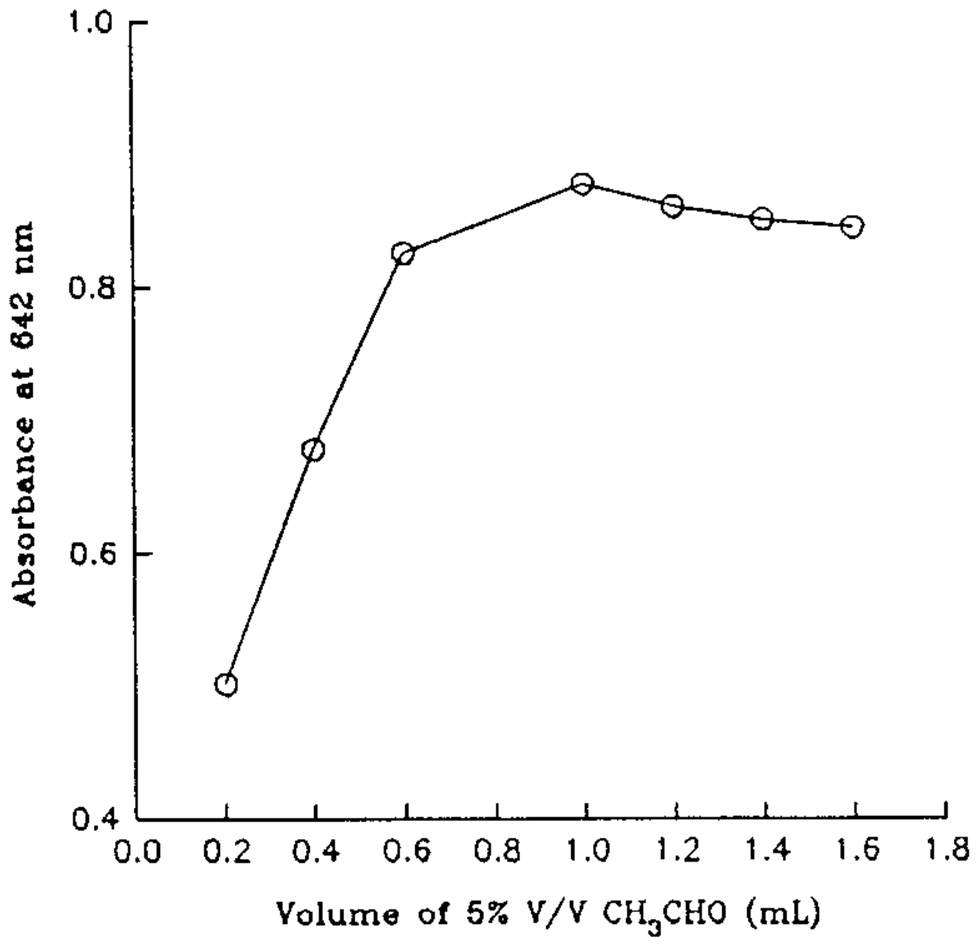

Fig. 2: Effect of volume of 5% v/v acetaldehyde on the absorption intensity of piperazine (8 $\mu g.mL^{-1}$) with DBMQ interaction product.

Similarly, the effect of various amounts of DBMQ on the intensity of the colored product was investigated. Results in Figure 3 revealed that optimum volume of 0.2% DBMQ is 1.2 mL. The effect of heating time was also studied by following the color development, in a boiling water bath at different time intervals. Absorption intensity was maximum after heating the reaction product for one minute. Further heating time decreased the intensity (Figure 4).

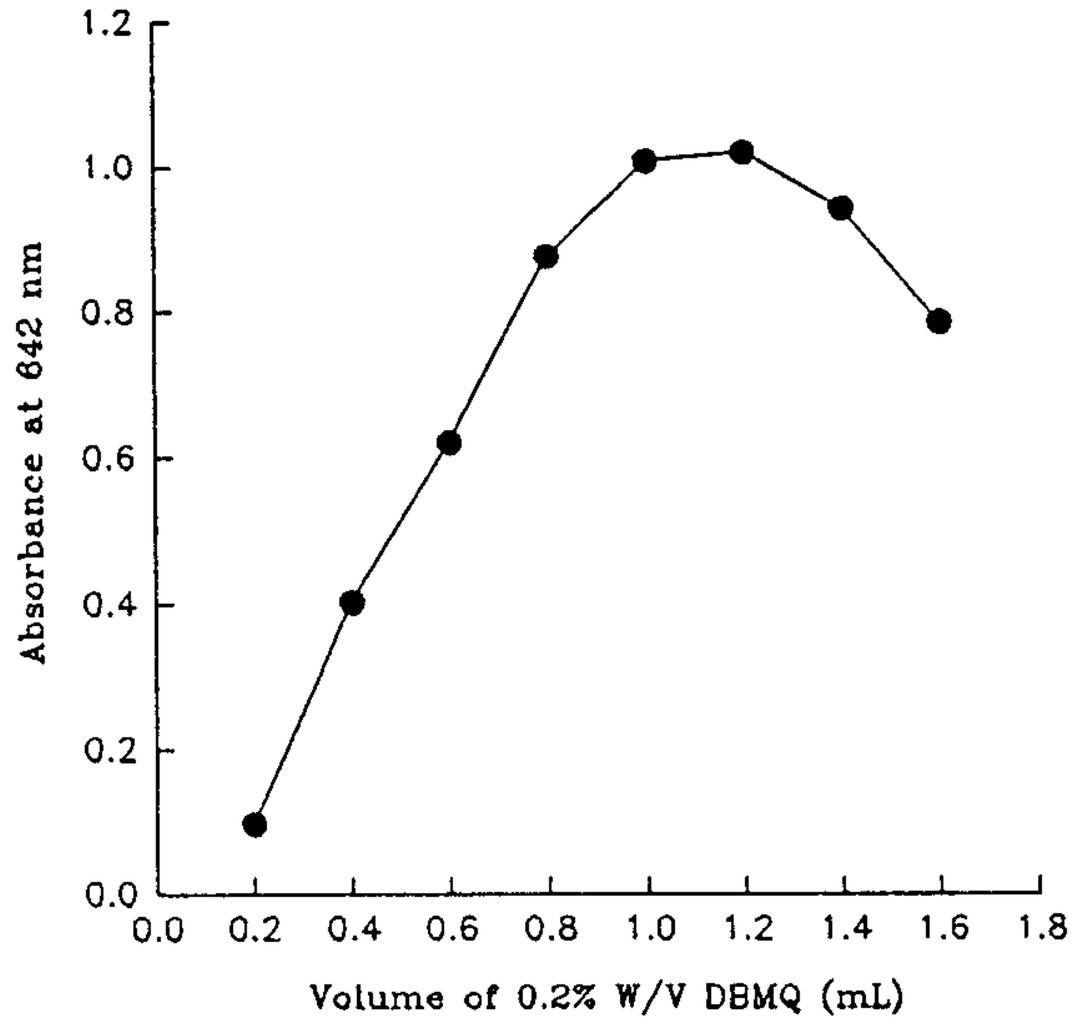

Fig. 3: Effect of volume of 0.2% methanolic solution of DBMQ on the absorption intensity of piperazine (8 $\mu g.mL^{-1}$) iinteraction product.

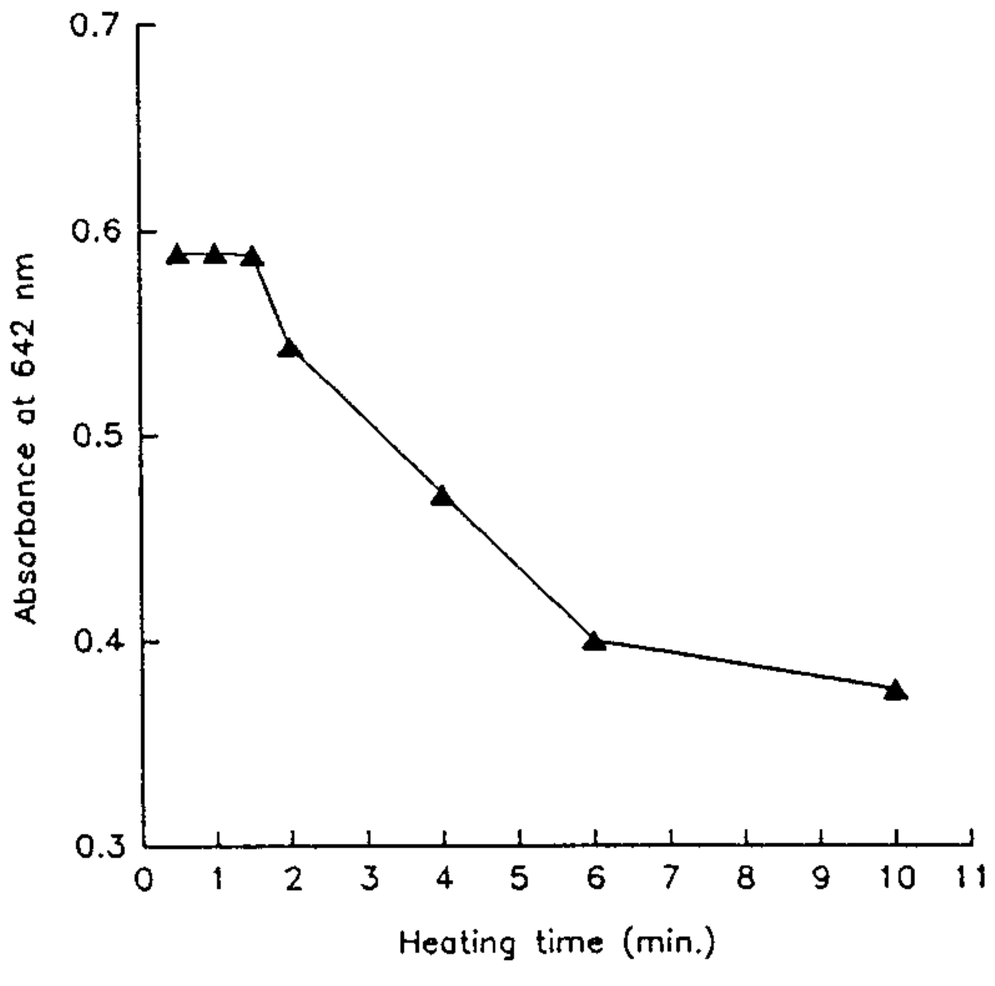

Fig. 4: Effect of heating time on the absorption intensity of piperazine (5 $\mu g.mL^{-1}$) with DBMQ interaction product.

Different solvents were tested as indicated in Table 1. Decolorization of the blue color occurred when using dioxane, while a relatively low absorption intensity was obtained with water and dimethylformamide. Other studied solvents were almost identical, therefore ethanol is selected as the diluting solvent.

For the liberation of base in situ from different piperazine salts (phosphate, citrate and tartrate), 0.01 N alcoholic sodium hydroxide solution was used. Different volumes of the alkali were tested indicating that a volume of 0.4 mL is quite sufficient. The stability of the colored product was evaluated by recording its absorption at 642 nm and was found to be stable for at least one hour at room temperature.

Under the studied assay parameters, regression analysis of Beer's plot gave good correlation. Linear concentration ranges, regression equations and respective regression coefficients for all the studied piperazines are listed in Table 2. To perform precision of the proposed method, eight replicate determinations were done on 5 μg piperazine per mL giving relative standard deviation not exceeding 1.6%.

Table 1: Effect of different solvents on the absorption intensity of piperazine : acetadehyde : DBMQ reaction product

Solvent	λ_{max}, nm	Absorbance*
Ethanol	642	0.491
Methanol	638	0.474
Isopropanol	640	0.458
Acetone	631	0.494
Acetonitrile	637	0.459
Dimethyl formamide	637	0.412
Dimethylsulfoxide	637	0.474
Water	636	0.330
Dioxane	decolorization completely	

* Final concentration of piperazine: 5 μg ml^{-1}.

Table 2: Statistical data for analysis of piperazine and its salts using DBMQ

Piperazine salt	Concentration range, μg ml^{-1}	r	a	b	ϵ
Piperazine hydrate	2-8	0.9963	0.092	0.110	2.50×10^4
piprazine citrate	2-10	0.9973	0.071	0.085	6.37×10^4
piprazine phosphate	2-10	0.9954	0.091	0.094	1.56×10^4
piprazine tartrate	2-9	0.9931	0.043	0.098	--

Table 3: Analysis of piperazine in different pharmaceutical formulations by the proposed and official methods.

Pharmaceutical formulation	Amount taken mg	% Found * ± SD		t[a]	F[b]
		Proposed method	Official method		
Parazine tablets[#]	25	98.32±0.28	98.02±0.34	1.52	1.48
Coliurinal efferevescent granules	50	96.77±1.68	95.63±1.89	1.01	1.26
piperazine citrate syrup[$]	50	99.96±1.38	98.20±1.93	1.66	1.96

* Average of 5 determinations.
# Reference (1).
$ Reference (2).
a Tabulated t for 8 degrees of freedom at P=0.05 is 3.83.
b Tabulated F for (4,4) degrees of freedom at P=0.05 is 6.39.

The suggested method was applied for the determination of piperazine in various pharmaceutical formulations. Results of the analysis show accuracy and precision of the proposed method (Table 3). Good agreement was found between the results of analysis of the studied drug by the proposed and official method. By applying the student's t-test and F-test, no significant difference is observed between the performance of the two methods with respect to accuracy and precision.

In conclusion, simplicity, quantitative yields and short reaction time are common characters of the proposed method. These are highly desired qualities for any successful analytical method.

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التعيين الضوئى للبيبرازين بإستخدام ٣،٥ ثنائى البروم-٢-ميثيل-بارا-بنزوكينون

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يتم التعيين الكمى للبيبرازين بطريقة ضوئية إما فى العقار النقى أو فى أشكاله الصيدلية.

تعتمد الطريقة على تكوين الفينيل أمين لهذا المركب بتفاعله مع الأستيالدهايد ويتفاعل الاينامين الناتج مع ثنائى البروم-٢-ميثيل-بارا-بنزوكينون يتكون لون أزرق غامق يتم قياسه عند ٦٤٢ نم.

ثنائى البروم-ميثيل-بارا-بنزوكينون تم تحضيره بطريقة الأكسدة المحورة بإستخدام اكسيد الكروم فى خليط من حمض الخليك والاسيتونيتريل (١:٩). ينطبق قانون بيرلامبرت مع معدل التركيزات من ٢-٨ ميكروجم لكل مل للبيبرازين.

لقد تمت دراسة كل العوامل المؤثرة على التفاعل للحصول على أنسب الظروف. الطريقة المقترحة بسيطة ، سريعة ، حساسة ودقيقة وممكن تطبيقها بنجاح لتحليل بعض الأشكال الصيدلية للبيبرازين مثل الأقراص والحبيبات الفوارة والشراب.

ونتائج التحليل متوافقة تماما مع نتائج الطرق الدستورية.

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