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HAEMATOXYLIN-PERMANGANATE REAGENT FOR THE SPECTROPHOTOMETRIC DETERMINATION OF SOME THIOL DRUGS

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تم استنباط طريقة طيفية جديدة وسريعة وذات حساسية عالية لتقييم ستة من مركبات الثيول الدوائية. وتعتمد هذه الطريقة على الاكسدة التلقائية لمجموعة الثيول باستخدام تركيز ضئيل وثابت من برمنجنات البوتاسيوم. ويتبع ذلك تفاعلا سريعا للزيادة من البرمنجنات مع مادة الهيماتوكسيلين فى وسط مائى متعادل. يتأكسد الهيماتوكسيلين إلى المادة الملونة "هيماتين" التى يمكن قياسها عند طول موجى ٥٥٨ ن.م. وتم الحصول على علاقة خطية بين تركيز المادة الدوائية والنقصان فى قراءة الامتصاص الضوئى. وتم تطبيق الطريقة لتقييم المركبات المدروسة سواء فى صورتها النقية أو فى بعض التركيبات الصيدلية. وكانت النتائج متوافقة تماما مع الطرق المنشورة سابقا. بالإضافة إلى هذا ، تم تطبيق الطريقة المقترحة كطريقة دالة على الثبات بالنسبة لمركبات الثيول الدوائية المدروسة.

A new rapid and sensitive spectrophotometric method was developed for the determination of six thiol pharmaceutical compounds. The method is based on the spontaneous oxidation of the thiol group with a constant small concentration of potassium permanganate followed by the rapid reaction of the excess permanganate with haematoxylin reagent in neutral aqueous medium. Haematoxylin is oxidized to the coloured chromogen, haematein which was measured at 558 nm. A linear relationship was obtained between drug concentration and the decrease in absorbance readings. The method was applied for the determination of the investigated compounds either in authentic powders or in some dosage forms. Good agreement was obtained with the reported methods. In addition, the suggested method was tried as stability indicating assay for the investigated thiol drugs.

INTRODUCTION

Thiol pharmaceutical compounds are considered to be medicinally important because of various therapeutic applications such as antihypertensive, antibacterial, antirheumatic and antidote for metal poisoning[1,2]. For the determination of thiols, several spectrophotometric methods have been reported using different reagents. Examples of these reagents are phosphotungstic acid[3,4], molybdic acid in sulphuric acid[5], chloranil[6], crystal violet-antimony hexachloride[7], 3-chloro-1,4-naphthoquinone[8], 2,6-dichloroquinone-4-chlorimide[9] and 4-chloro-7-nitrobenzo-furazan[10]. There are also some reported spectrophotometric methods for the determination of individual thiol pharmaceutical compound; e.g. captopril[11-15], D-penicillamine[16,17], thiosalicylic acid[18], thiobarbituric acid and tiopronin[19].

Haematoxylin [7,11b-dihydrobenz-(b)-indeno-[1,2-d]-pyran-3,4,6a,9,10-6(h)-pentol] is a catechol derivative from logwood (*Haematoxylin Campeachianum*)[20]. Originally, it is an important stain in biology[21]. It or its oxidized form has been widely used for the detection and determination of several metal ions[20,22-28]. Also, it has been used as a metallochromic indicator[29] and as an indicator for the determination of phosphorus[30] and

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fluorine[31]. Recently, it has been introduced in pharmaceutical analysis for the spectrophotometric determination of benzthiazide and hydrofluoromethiazide[32].

In this work, six pharmaceutical compounds having chemically active thiol group and utilized for various therapeutic applications were determined spectrophotometrically using haematoxylin-potassium permanganate as a new reagent.

EXPERIMENTAL

Instruments

- Perkin-Elmer Lambda-3B,UV/VIS Spectrophotometer connected with Perkin-Elmer R100 A recorder (USA), with two matched 1 cm quartz cells.
- Uvidec 320 Spectrophotometer (Japan), with two matched 1 cm quartz cells.

Reagents and materials

- Potassium permanganate (El-Nasr Chemical Co., Egypt), 0.02 mg/ml in distilled water.
- Haematoxylin (Aldrich, USA), 1 mg/ml is freshly prepared in methanol. Solution should be light yellow in colour.
- Ammonium Acetate (El-Nasr Chemical Co., Egypt), 0.5 % w/v solution in distilled water.
- The following authentic drug samples were used as working standards: captopril (Squibb, Egypt); sulphathiourea (Bayer Co., Germany); D-penicillamine (Sigma Co. St. Louis, USA); thiosalicylic acid and tiopronin (Aldrich Co., USA) and thiobarbituric acid (BDH, Chemicals Ltd. Boole, England).

Dosage forms

- Capoten tablets (Squibb, Egypt): contain 25 mg captopril per tablet.
- Capozid tablets (Squibb, Egypt): contain 50 mg captopril and 25 mg hydrochlorothiazide per tablet.
- Bendonal tablets (Alex. Co., Egypt): contain 500 mg sulphathiourea per tablet.
- Badional tablets (Bayer Leverkusen, Germany): contain 500 mg sulphathiourea per tablet.
- Artamine capsules (Biochemie Co., Austria): contain 250 mg D-penicillamine per capsule.
- Simulated tablet preparation was made for tiopronin by mixing 100 mg of the drug with about 0.2 g of each of gum acacia, magnesium stearate and lactose then finely powdering.

Preparation of drug standard solutions

Stock solutions (0.1% w/v) of each of the studied thiol compounds were prepared separately in distilled water for captopril, D-penicillamine and tiopronin; and in methanol for sulphathiourea, thiosalicylic acid and thiobarbituric acid. These solutions were diluted quantitatively with distilled water to obtain the proper concentration.

Preparation of drug sample solution

Twenty tablets or the content of ten capsules were finely powdered and weighed. Into a 50-ml volumetric flask, an accurately weighed portion of the powder equivalent to about 50 mg of the thiol drug was transferred. Volume was completed with distilled water in cases of capoten tablets, capozide tablets, tiopronin simulated tablets and artamine capsules; and with methanol in cases of bendonal and badional tablets. The resulting mixture was shaken well and filtered off, rejecting the first 5-10 ml of the filtrate. This prepared solution was diluted quantitatively with distilled water to obtain the proper concentration.

General procedures

Into a 10-ml volumetric flask, 1.0 ml of the standard or sample solution was pipetted. One ml of potassium permanganate solution was accurately measured, added and mixed. Half ml of haematoxylin methanolic solution was added, mixed and immediately the volume was completed with ammonium acetate solution. The difference in absorbance was measured within 5 minutes at the maximum wavelength of 558nm against blank solution prepared similarly using

1.0 ml of distilled water instead of the sample or standard solution.

Stability indicating assay procedure

An aqueous solution of each of captopril, D-penicillamine and sulphathiourea; 60, 50 and 80 mcg/ml respectively was prepared in a 100-ml volumetric flask and allowed to stand in a constant boiling water bath. At different time intervals up to two hours (0, 10, 20 ... 120 minutes), one ml of this solution was transferred to a 10-ml volumetric flask and the general procedure was applied. Percentages of the remaining drug after each time interval were calculated. A relation between these percentages and time is graphically obtained.

RESULTS AND DISCUSSION

In this work, six of the pharmaceutical thiol compounds were determined by a spectrophotometric method. The method is based on the reaction of the thiol compound, either in pure form or in pharmaceutical dosage form, with a constant sufficient amount of potassium permanganate. The excess permanganate was then allowed to react with haematoxylin reagent in neutral aqueous medium to produce a coloured chromogen which has two wavelengths of maximum absorption; at 440 and at 558 nm, Fig. 1. Wavelength of 558 nm was selected for absorbance measurements being of higher intensity and bathochromically shifted away of any expected interference. The proposed method is based on the presence of the thiol group which is reported to be essential for the pharmacological activity of these compounds[33].

Permanganate has not been used greatly in analytical work with thiols, especially in quantitative procedures. This is because of uncertainty about the reaction stoichiometry[34]. In mild conditions, the oxidation product is the disulphide. While, the oxidation may proceed beyond the disulphide stage depending on the molecular environment of the --SH group, the nature of the oxidizing agent, the pH, the concentration of the reactants and the presence of metal ions[35]. Accordingly, because of the mild conditions of the suggested reaction including the very low concentration of the

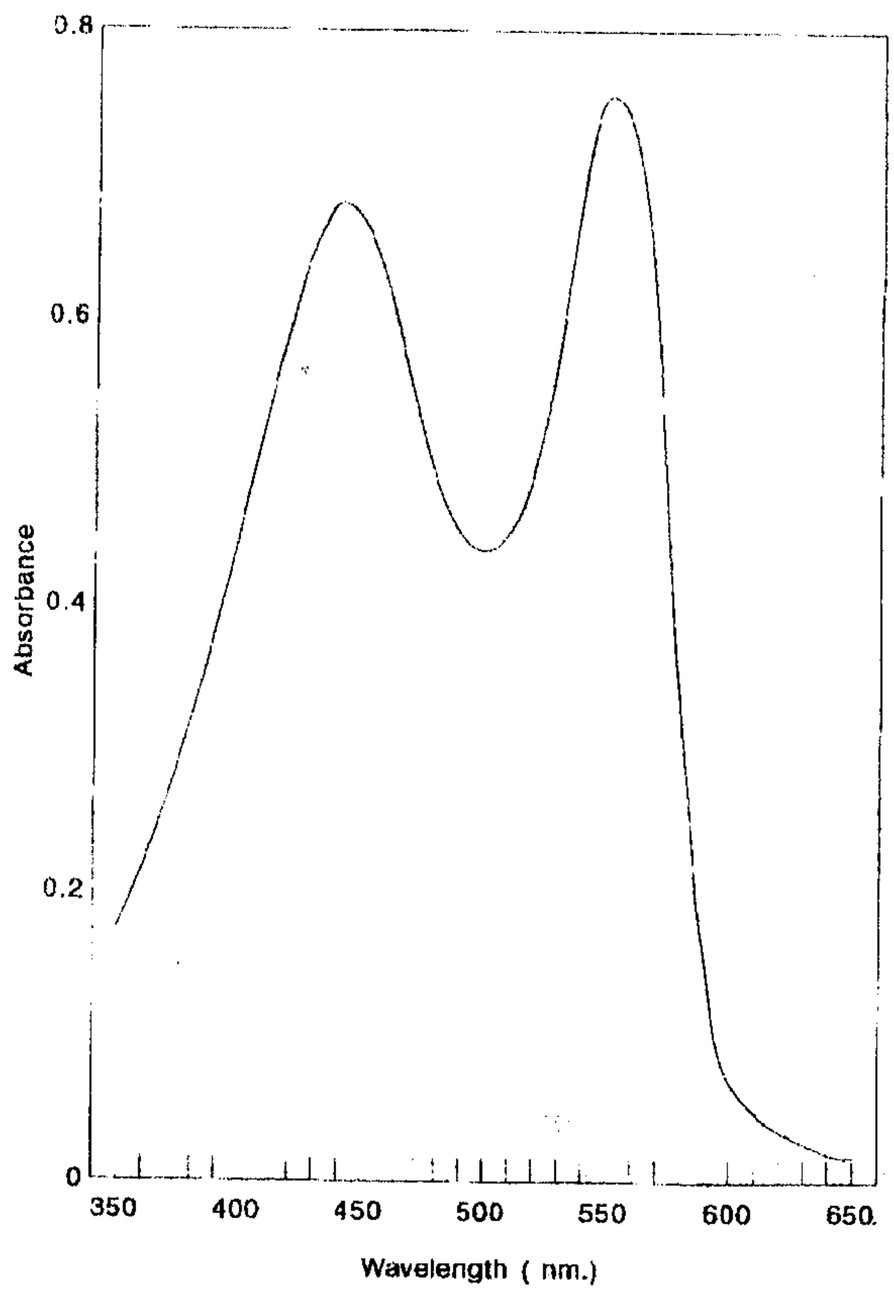


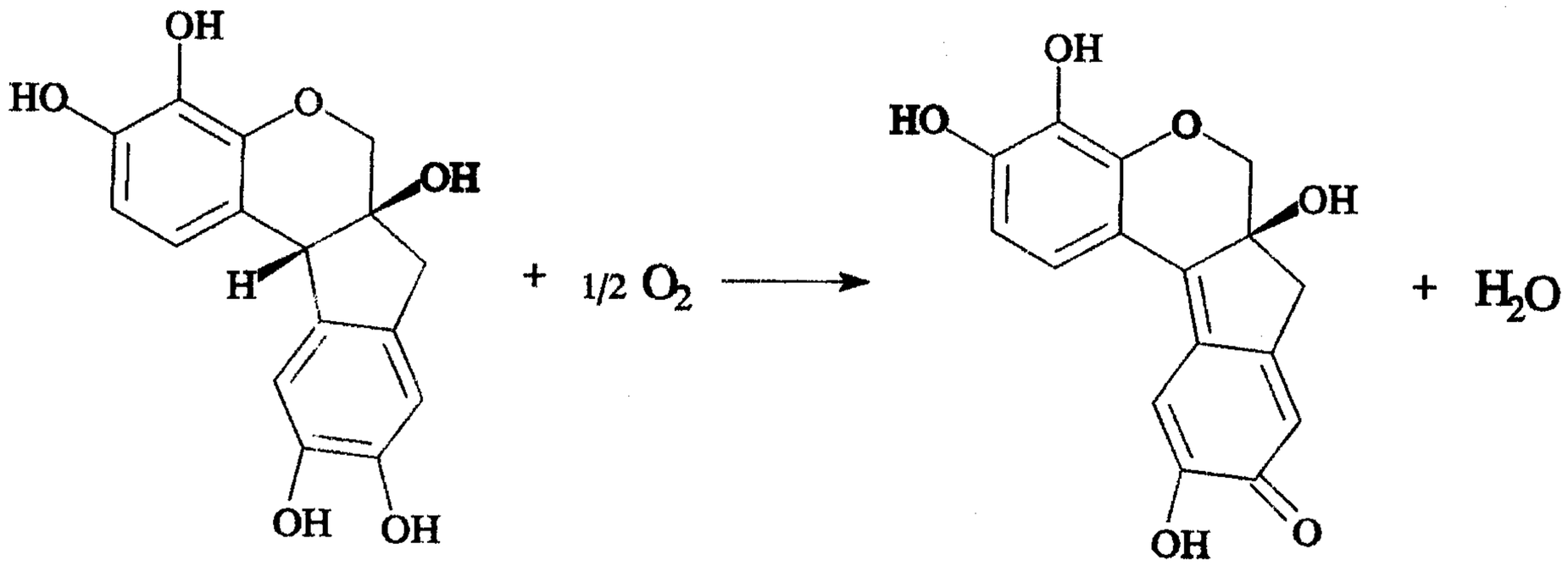
Fig. 1: Absorption spectrum of oxidized haematoxylin.

reactants, the neutral medium and the absence of metal ions, the reaction product may suggested to be the disulphide presented by the following equation:

$$2RSH + \tfrac{1}{2} O_2 \rightarrow RSSR + H_2O$$

As it is well known, haematoxylin by oxidation can produce a coloured product, haematein [36]. So, the oxidation of haematoxylin by the excess permanganate may be presented by Scheme 1. Both of these two reactions were found to be very rapid and spontaneous at room temperature. The intensity of the produced chromogen (haematein) was found to be thirty times as much as that of the free permanganate colour. This indicates the very high sensitivity of the method. In addition, the use of the specified very small concentration of potassium permanganate increases the specificity of the reagent towards the thiol compounds.

Oxidants, other than permanganate, e.g ferric chloride, potassium ferricyanide,



Haematoxylin Haematein

Scheme 1: Oxidation of haematoxylin

potassium persulphate, iodine and N-bromo-succenimide, were tried. Permanganate was the best regarding rapidity of the reaction and sensitivity of the produced chromogen.

Haematoxylin aqueous solution produces a gradual change in colour due to oxidation. Therefore, it was used in methanolic solution which has a slight yellow colour and relatively good stability. However, solution of this reagent should be daily freshly prepared. The optimum concentration of haematoxylin reagent was determined by trying various increasing concentrations of the reagent. At the reaction conditions, 0.05 mg/ml final concentration was found to be optimum. Blank experiment using haematoxylin with no permanganate gave no absorbance reading at 558 nm.

The produced chromogen was found to have the best intensity and stability in neutral aqueous medium. Slightly alkaline medium (pH > 8) produces a brown turbidity due to the production of manganese dioxide as a reduction product of permanganate. In slightly acidic medium (pH < 6), the chromogen is transformed into an orange yellow coloured product with lower intensity. Therefore, the neutral medium is essential. Other solvents like methanol, ethanol, isopropanol, acetone and dioxane, when used as diluents produce lower colour intensity and lower stability of the chromogen. The coloured chromogen was found to be nonextractable by water immiscible solvents like benzene and halogenated hydrocarbons.

Colour of the produced chromogen was found to be slightly unstable with time and successive increase in absorbance readings was observed. Many attempts were done to overcome this drawback. Reducing agents like sodium metabisulphite bleaches the chromogen colour completely; may be due to the full reduction of haematein. Ammonium acetate solution 0.5 % w/v in distilled water was selected to be effective in this regard and reproducible results were obtained when the absorbance was measured within five minutes after preparation. No better results was obtained by 1, 2, 3, 4 and 5% solution, while 0.1, 0.2 and 0.3% solution were less effective.

The suggested method was applied for the determination of the six investigated thiol drugs. Linear correlation between concentration and the decrease in absorbance was obtained at the specified reaction conditions. Linear ranges and other statistical data used for the quantitation of the investigated compounds are presented in Table 1. The suggested method was applied for the analysis of the authentic samples and some

Table 1: Spectral characteristics of the investigated thiol drugs.

Compound	Linear range mcg/ml	Intercept (a)	Slope (b)	Correlation coefficient	ϵ_{max} x 10^4 $L.Mol^{-1}cm^{-1}$
Captopril	1-10	0.0046	0.106	0.9997	2.30
D-Penicillamine	1-8	-0.0062	0.144	0.9998	2.15
Sulphathiourea	2-15	0.0101	0.053	0.9991	1.23
Tiopronin	1-6	-0.0047	0.144	0.9995	2.35
Thiobarbituric acid	2-12	-0.0094	0.088	0.9999	1.27
Thiosalicylic acid	5-20	0.0088	0.055	0.9995	0.85

Table 2: Analysis of some thiol drugs in pure forms and in pharmaceutical preparations.

Compound	% Found* ± SD		t***	F***
	Proposed method	Reported method**		
Captopril				
Bulk drug	98.51±0.52	98.48±0.60	0.065	1.331
Capoten tablets	99.23±0.45	99.09±0.36	0.421	1.562
Caposide tablets	98.04±0.76	97.96±0.71	0.133	1.146
Sulphathiourea				
Bulk drug	100.02±0.12	99.69±0.17	0.490	2.007
Bendonal tablets	99.36±0.66	98.39±0.44	2.114	2.250
Badional tablets	99.85±0.34	99.68±0.66	0.397	3.768
D-penicillamine				
Bulk drug	99.06±0.66	99.06±0.34	0.000	3.768
Artamine capsules	100.02±0.55	99.66±0.59	0.773	1.151
Tiopronin				
Bulk drug	99.89±0.33	100.23±0.63	0.828	3.645
Simulated tablets	100.44±0.66	99.44±0.74	1.747	1.257

* Mean of 3 determinations.

** For captopril, Ref. 15 & 37. For sulphathiourea, Ref. 38. For D-penicillamine, Ref. 37 p1022. For tiopronin, Ref.39.

*** Tabulated value for t-test = 2.132 and for F-test = 9.280 at P = 0.05

dosage forms. Results of the proposed method were in good agreement with those of the reported methods Table 2. No interference was observed due to the common dosage form additives and excepients. Also, no interference was found due to hydrochlorothiazide present with captopril in capozid tablets.

In the suggested procedures, it was observed that no interference occurred due to the presence of the reaction product; the disulphide.

Therefore, attempts were done for stability indicating assay; specially it is well known that thiols in solutions upon shelf storage undergo an easy auto oxidation degradation reaction with the formation of the disulphide[35]. The suggested method was applied in this regard using captopril, sulphathiourea and D-penicillamine as examples. Constant boiling water bath was used to accelerate the degradation of the thiols. Results shown in Fig. 2, indicate the suitability of the proposed method as stability indicating assay.

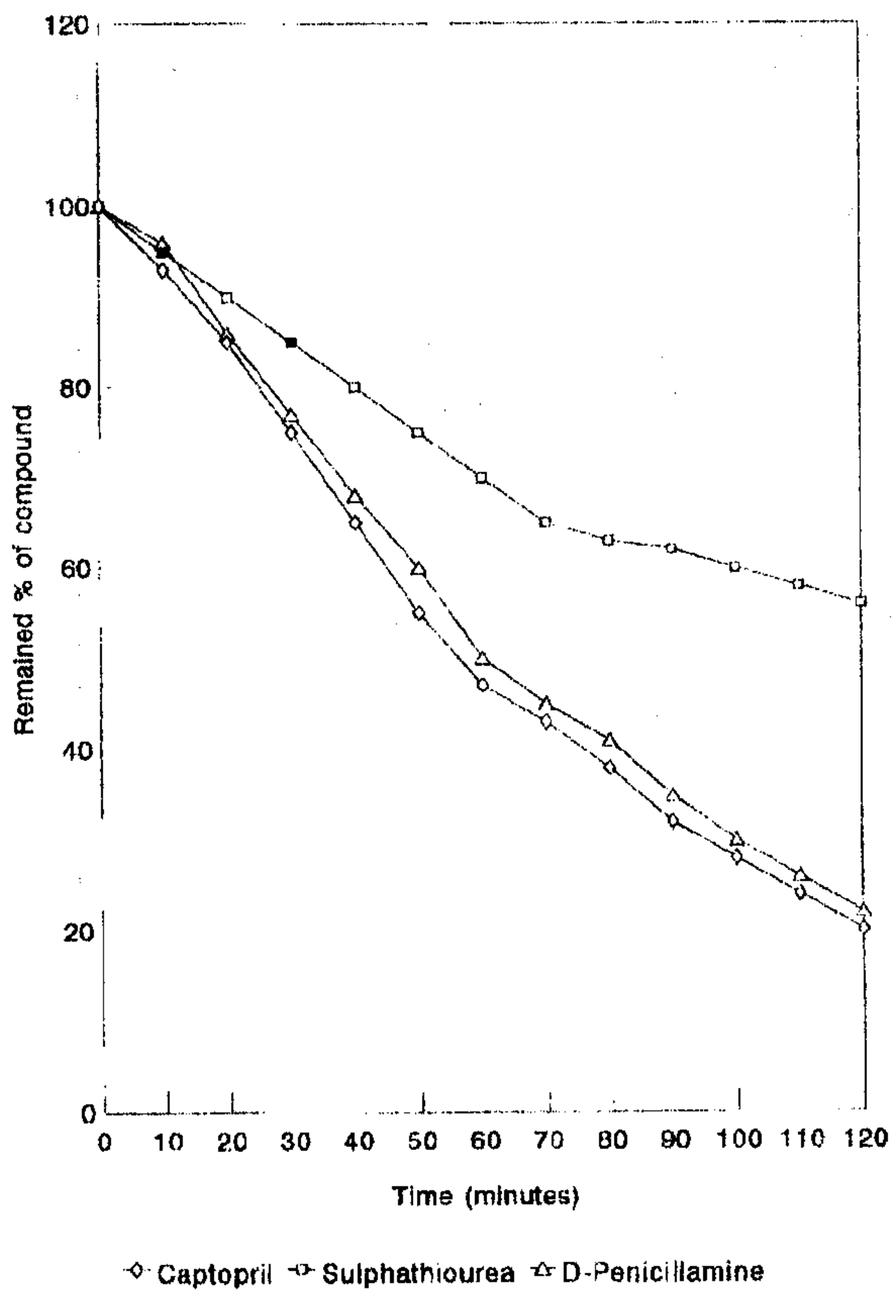


Fig. 2: Stability indicating assay of some thiol drugs.

In conclusion, the described method in this work has the advantages of simplicity, rapidity and high sensitivity; in addition to the extension of the use of haematoxylin as a relatively new spectrophotometric reagent in the field of pharmaceutical analysis.

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