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SPECTROPHOTOMETRIC DETERMINATION OF SOME PHARMACEUTICAL THIOLS USING 2,6-DICHLORO-QUINONE-4-CHLORIMIDE*

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ABSTRACT: *The reaction of thiols with 2,6-dichloroquinone-4-chlorimide (DQC) at pH 7-8 was applied to the quantitative determination of 9 thiol compounds related to different classes (aliphatic, aromatic and heterocyclic). The aliphatic thiols yielded a yellow color (λ_{max} 435 nm) aromatic thiols yielded an orange color (λ_{max} 498-503 nm), sulphathiourea, 2-thiobarbituric acid and thiacetazone yielded violet colors (λ_{max} 540-550 nm). Job's plots indicate a 1:1 ratio of thiol to reagent. Beer-lambert's law is adhered to over the following ranges 2-4 μg ml^{-1} (for 2-thiobarbituric acid), 3-6 μg ml^{-1} (for thiosalicylic acid), 4-18 μg ml^{-1} (for thiomersal), 2-14 μg ml^{-1} (for thiacetazone) 10-70 μg ml^{-1} (for captopril), 5-25 μg ml^{-1} (for dimercaprol), 10-40 μg ml^{-1} (for thioglycerol) and 5-30 μg ml^{-1} (for tiopronin). Common tablet excipient as well as additives in parenteral solutions were found not to interfere. The results of analysis of pure drugs and their dosage forms by the proposed method are in good agreement with those obtained by official and reported methods.*

INTRODUCTION

A number of drugs which contain thiol or an another group that gives thiol on tautomerism or hydrolysis are commercially available and are utilized in various therapeutic applications such as antibacterial[1], antithyroid[2], antidote to metal poisoning[2], antihypertensive[1], preservative[2], hepatoprotectant[2], antirheumatic[3], and tuberculostatic agents[1].

Numerous methods are available in the literature for the quantitative analysis of thiol drugs. These methods include titrimetry[4-6], TLC[7], GLC[8], HPLC[9-13], fluorimetry[14-16], spectrophotometry[17-20], potentiometry[21], polarography[22,23] and coulometry[24,25].

The present investigation was undertaken to develop a spectrophotometric method for the quantitative analysis of nine thiol derivatives of pharmaceutical interest, based on the interaction of the thiol group with DQC to yield colored quinone sulfenimines.

EXPERIMENTAL

Instruments:

- Perkin-Elmer Lambda-3ß, UV/VIS spectrophotometer connected with Perkin-Elmer R 100 A recorder (USA).
- APW 9418 pH meter (Pye Unicam Cambridge, UK).

Reagents and materials:

2,6-Dichloroquinine-4-chlorimine (DQC) was obtained from Fluka AG, CH-9470 Buchs England. Stock solution (0.5 % w/v) in absolute ethanol (spectroscopic grade) was prepared. This solution was stable for 4 weeks at about 4°C.

Captopril and hydrochlorothiazide were obtained from Squib-Egypt. Thiosalicylic acid, dimercaprol, thioglycerol and tiopronin were obtained from Aldrich Co., USA. Thiacetazone and thiomersal were obtained from Sigma, USA.

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Thiobarbituric acid was obtained from BDH Chemicals Ltd, Poole, England. Sulphathiourea was obtained from Bayer Co., Germany. They are analyzed according to some reported methods and their purities are listed in Table 4.

Dosage forms:

1- Capoten tablets, Squib-Egypt, contain 25 mg of Captopril per tablet.
2- Capozide tablets, Squib-Egypt, contain 50 mg of captopril and 25 mg of hydrochlorothiazide per tablet.
3- Bendonal tablets, Alex. Co., contain 500 mg Sulphathiourea per tablet.
4- Hydrocare soaking solution, Allergan, Westport, Co. Mayo, Ireland contains 0.002 % w/v thiomersal and 0.003 % w/v alkyl triethanolammonium chloride.

Synthetic mixtures:

1- Simulated tablet preparations were made for both thiacetazone and tiopronin. Equal quantities of each drug, together with gum acacia, gum tragacanth, magnesium stearate and lactose were mixed and finely powdered.
2- Simulated dimercaprol injection was prepared according to BP 1985[26].

All other chemicals and solvents were of analytical grade reagent.

Solutions:

1- **Buffer Solutions:** Phosphate buffer of pH 7 and 7.5; tetraborate buffer of pH 8, 8.5, 9 and 9.5 were prepared[27].
2- **Preparation of standard solution:** A stock solution (1 mg ml^{-1})of each drug was prepared in ethanol. This solution was diluted quantitatively to obtain the specified concentration range (Table 2) for each drug.
3- **Tablet or synthetic mixture sample solution:** Weigh and powder 20 tablets. Into 100 ml volumetric flask transfer an accurately weighed quantity of the powdered tablets or synthetic mixture, equivalent to 100 mg of the drug. Shake with 30 ml ethanol and complete to volume with the same solvent. Filter and discard the first 10 ml of the filtrate, dilute each solution quantitatively to obtain the following concentrations: 6 μg/ml for sulphathiourea; 20 μg/ml captopril; 10 μg/ml for thiacetazone or 20 μg/ml for tiopronin.
4- **Preparation of dimercaprol injection sample:** Extract 2.0 ml of simulated dimercaprol injection two times each with 5 ml of distilled water. Dilute the collected extracts to 100 ml with ethanol. Transfer 1.0 ml of this solution to 10 ml volumetric flask and complete to volume with ethanol.

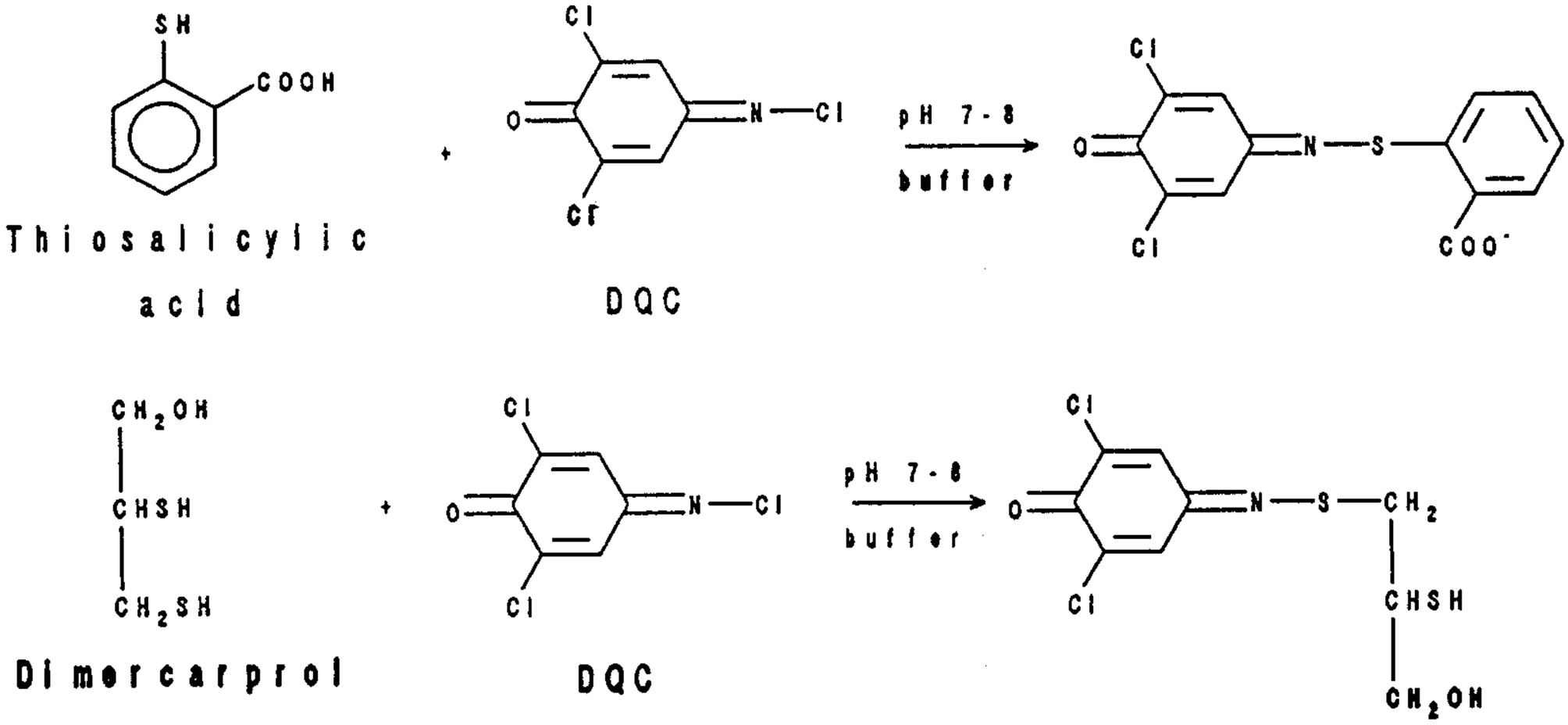
General assay procedure:

Into 10 ml volumetric flask, transfer 1 ml of the assay solution, add the appropriate quantities of water, buffer and reagent (Table 1), heat in a water bath at 60°C for about 8 minutes or leave for the optimum reaction time. Complete to volume with the suitable solvent (Table 1). Measure the absorbance of the resulting solution, within 30 min, at the specific λ_{max} (Table 1) against a reagent blank prepared similarly. For Hydrocare solution transfer 3 ml into 10 ml volumetric flask, continue as described and prepare a standard aqueous solution of thiomersal containing 2 μg/ml. Use 3 ml of the last solution for color development as previously described.

RESULTS AND DISCUSSION

It has been reported that some substituted heterocyclic thiol compounds: thiouracil, propyl thiouracil and methimazole were spectrophotometrically determined via the reaction with DQC[28-30]. One of these methods involved the extraction of the formed yellow dye with chloroform and measuring the absorbance at 410 nm[28]. Other method involved the formation of charge transfer complex[30].

In the present work, DQC was used for the spectrophotometric determination of nine drugs which contain thiol or another group that gives thiol on tautomerism or hydrolysis. The drugs investigated belong to different classes (aliphatic, aromatic and heterocyclic). This reaction was found to proceed via the formation of quinone sulfenimines[31]. Accordingly; the suggested mechanism is illustrated in scheme 1.



Scheme 1
Pathway of the reaction of thiols with DQC to form quinone sulfenimines

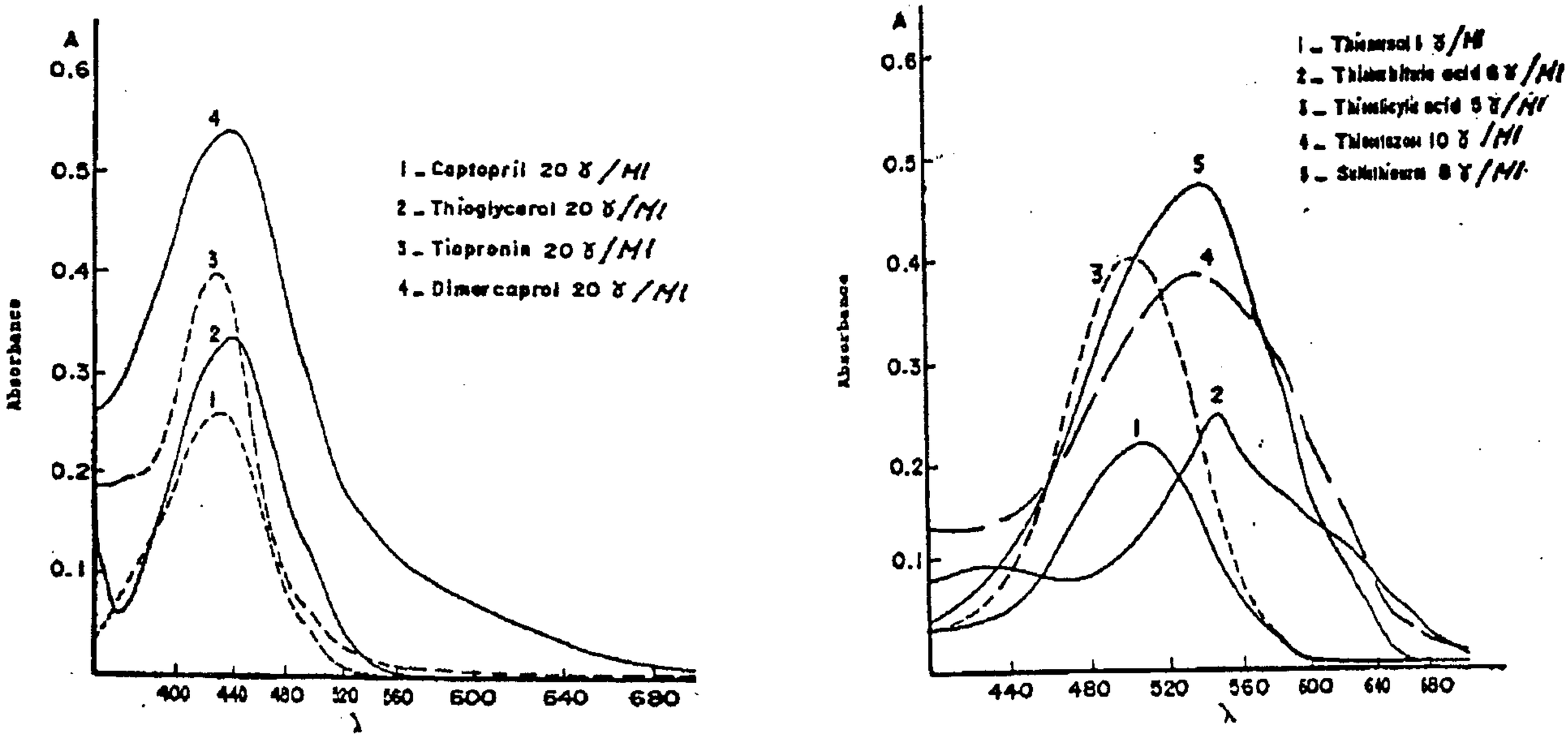


Fig. 1: Absorption spectra of the chromophores resulting from the interaction of (a) aliphatic thiols, (b) aromatic thiols, with DQC.

Table 1: Summary of the assay parameters recommended for the spectrophotometric determination of thiols by DQC.

Compund	Volume of distilled water added (ml)*	pH of buffer added	Reagent		Heating time at 60°C (min.)	Reaction time (min.)	Solvent	Colour obtained	max. (nm.)
			Conc. (%)	Volume (ml)					
Sulphathiourea	2	7.5	0.05	0.1	-	10	Isopropanol	Violet	542
2-Thiobarbituric acid	2	7.5	0.3	0.2	8	-	Isopropanol	Violet	545
Thiomersal	2	8.0	0.5	0.1	-	25	Isopropanol	Orange	499
Captopril	-	-.-	0.5	0.2	-	10	Ethanol	Yellow	435
Dimercaprol	1	7.5	0.5	0.2	-	10	Water	Yellow	435
Thioglycerol	1	7.0	0.4	0.2	-	5	Water	Yellow	435
Tiopronin	-	8.0	0.2	0.2	-	5	Methanol	Yellow	435
Thiosalicylic acid	-	8.0	0.3	0.1	-	10	Isopropanol	Orange	502
Thiacetazone	-	8.0	0.2	0.2	-	10	Ethanol	Violet	545

* Distilled water is added before the addition of buffer solution except in case of sulphathiourea where water is added after the reaction time.

Table 2: Spectral characteristics and quantitative parameters for the reaction products of thiols with DQC.

Compound	λ_{max} nm.	$\epsilon x10^3$ $L.mol^{-1}cm^{-1}$	Linear range μgmL^{-1}	a*	b*	r*
Sulphathiourea	545	12.687	3-8	-0.073	0.065	0.9998
2-Thiobarbituric acid	542	9.682	3-8	0.061	0.056	0.9995
Captopril	435	2.853	10-70	0.006	0.012	0.9996
Thiomersal	502	17.153	4-18	0.033	0.036	0.9990
Dimercaprol	435	3.734	5-25	0.080	0.025	0.9995
Thioglycerol	435	2.195	10-40	-0.007	0.020	0.9990
Tiopronin	435	3.574	5-30	0.043	0.019	0.9999
Thiosalicylic acid	502	18.117	3-6	-0.085	0.137	0.9990
Thiacetazone	545	12.051	2-14	0.023	0.046	0.9993

* a= Intercept, b= Slope and r= Correlation coefficient.

The absorption curves of the resulting chromophores are shown in Figures 1 (a and b). The absorption maxima of the chromophores were found to be different according to the type of compounds: aliphatic compounds yielded a yellow colored chromogen (λ_{max} 430-440 nm), aromatic compounds yielded an orange colored product (λ_{max} 499-503 nm); sulphathiourea, 2-thiobarbituric acid and thiacetazone yielded violet colors (λ_{max} 545-550 nm). This chromophore classification agrees with previous reports[32]. The apparent molar absorptivities are in the range of 10^4 to $3x10^4$ $L.mol^{-1}$ cm^{-1} (Table 2).

By application of Job's plot of continuous variation to three representative compounds (one aliphatic monothiol, one aliphatic dithiol and an aromatic thiol compounds) a molar ratio of 1:1 was obtained in all cases.

Optimization of Variables:

Five factors were found to affect the intensity and stability of the resulting colors; reagent concentration, pH, solvent, temperature, order of addition of reagent and buffer. As shown in Table 1, the optimum concentration of reagent solution, required for maximum color intensity, differs according to the compound analyzed (0.05-0.5 % w/v). This may be explained by differences in the linear concentration ranges and the production of different colored compounds having different absorption maxima. The use of reagent concentration higher than 0.5 % w/v leads to the formation of an intensely colored blanks.

In general, the reaction takes place in a buffered neutral or slightly alkaline medium (pH 7-8), (Table 1), while in acidic medium no color was observed. With the exception of captopril (no buffer was needed), all other compounds were analyzed in presence of phosphate or tetraborate buffers, which increase the color intensity. Buffer solution with pH > 8 is not recommended owing to the formation of dark colored blanks. Buffer solution must be added and mixed with the sample before reagent addition, otherwise less intense colors were obtained. In addition; ammonia buffers must not be used since it reacts with the reagent[33].

In most cases, a red shift and improved stability of the chromophore were observed in presence of a suitable organic solvent. In some cases, it was necessary to add 2 ml of water before addition of buffer or after reaction time

Table 3: Effect of solvent on the absorption colour intensity of the reaction products of thiols with DQC.

Compound	Dioxan	Methanol	n-Propanol	Isopropanol	Ethanol	Water
Sulphathiourea	0.315	0.318	0.292	0.327	0.317	0.250
2-Thiobarbituric acid	0.416	0.377	0.385	0.406	0.378	0.443
Captopril	0.461	0.461	0.472	0.479	0.543	0.441
Thiomersal	0.364	0.384	0.314	0.409	0.323	0.438
Dimercaprol	0.328	0.299	0.322	0.284	0.330	0.331
Thioglycerol	0.390	0.270	0.400	0.380	0.350	0.400
Tiopronin	0.413	0.445	0.436	0.420	0.442	0.428
Thiosalicylic acid	0.429	0.503	0.484	0.627	0.602	0.507
Thiacetazone	0.270	0.188	0.453	0.435	0.493	0.140

Table 4: Analysis of the investigated thiols in bulk drug and dosage forms.

Compound (Dosage form)	Amount taken mg	% Found* ± SD		t***	F***
		Proposed	Reported**		
Captopril					
bulk drug	100	98.84±0.67	98.92±0.55	0.21	1.5
(Capoten tablet)	25/tab	99.42±0.90	100.25±0.62	1.69	2.31
(Capozide tablet)	50/tab	99.68±0.76	99.22±0.81	0.92	1.13
Sulphathiourea					
bulk drug	50	101.56±0.73	101.32±0.46	0.62	2.52
(Bendonal tablet)	500/tab	101.76±0.86	102.8±1.17	1.50	1.85
Thiomersal					
bulk drug	50	98.77±0.38	98.24±0.32	2.42	1.4
(Hydrocare solution)	0.02/ml	100.46±1.19	99.68±1.01	1.00	1.36
Dimercaprol					
bulk drug	100	98.20±0.35	98.14±0.55	0.21	2.4
(Dimercaprol injection)	50/ml	97.20±0.66	96.27±0.50	0.88	1.78
Thiacetazone	100	99.22±0.42	97.96±0.69	0.72	2.77
bulk drug	10/tab	100.98±0.68	101.02±0.62	0.36	1.20
(Simulated Thiacetazone tablet)					
Tioproin					
bulk drug	100	98.44±0.34	98.01±0.18	2.54	3.65
(Simulated Tiopronin tablet)	20/tab	98.46±1.16	97.30±1.81	1.07	1.25

* Mean of 5 determinations.

** Reference 34,37 for Captopril, 35 for Sulphathiourea, 36, 37 for Thiomersal, 37 for Dimercaprol injection, 38 for Thiacetazone and 39 for Tiopronin.

*** Tabulated value of t(8, 0.05)=3.83; F(4,4,0,05)=6.39.

to prevent precipitation of buffer salts. The effect of different solvents on the color development and stability was presented in Table 3.

With the exception of 2-thiobarbaturic acid, (heating is needed), all thiol compounds investigated react with DQC fairly rapidly on cold (Table 1). In these cases heating of the reaction mixture leads to the formation of unstable colors and dark blanks. In general the reaction product was found to be stable for at least 30 min. The optimum conditions for the reaction, color development and stability are summarized in Table 1.

A linear correlation was found between the absorbance and concentration for each compound. The concentration ranges, correlation coefficients, intercepts and slopes for the investigated thiols are presented in Table 2.

Application to bulk drugs and dosage forms analysis:

Table 4 shows the results obtained from the determination of thiol drugs in pure forms and in some of their dosage forms by adopting the proposed and the reported methods. Comparison with the reported methods revealed that the proposed method is equally accurate. The proposed method showed a good precision, the relative standard deviation observed for the analysis of the thiols listed in Table 4, do not exceed $\pm$ 1.8 %.

In conclusion, the method offers a relatively simple, sensitive and rapid means of analysis of pharmaceutical thiols comparable to pharmacopeial and reported methods.

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التعيين الضوئى لبعض الثيولات الصيدلية بإستخدام ٢،٦-ثنائى كلوروكينون-٤-كلوراميد

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تم تطبيق تفاعل الثيولات مع ٢،٦-ثنائى كلوروكينون-٤-كلوراميد للتعيين الكمى لتسعة مركبات ثيول من أقسام مختلفة (اليفاتية ، أروماتية وغير متجانسة الحلقة).

لقد تكون لون أصفر مع الثيولات الاليفاتية (عند ٤٣٥ نم)، لون برتقالى مع الثيولات الاروماتية (عند ٤٩٨ - ٥٠٣ نم) ولون بنفسجى مع السلفاثيوبوريا مع حمض ٢-ثيوباربتييوريك ومع الثياستيازون (عند ٥٤٠ - ٥٥٠ نم).

ينطبق قانون بيرلامبرت مع معدل التركيزات: ٢-٨ ميكروجم لكل مل حمض ٢-ثيوباربيتيوريك، ٣-٦ ميكروجم لكل مل حمض ثيوساليسليك، ٤-٨ ميكروجم لكل مل ثيوميرسال، ٢-١٤ ميكروجم لكل مل ثياستيازون، ١٠-٧٠ ميكروجم لكل مل كابتوبريل، ٥-٢٥ ميكروجم لكل مل داى ميركابرول، ١٠-٤٠ ميكروجم لكل مل ثيوجليسرول و٥-٣٠ ميكروجم لكل مل تيوبرونين.

لقد وجد أن صواغات الاقراص الشائعة ومضافات محاليل الحقن لاتتداخل مع طريقة التعيين.

الطريقة المقترحة لتعيين هذه العقارات سواء فى صورتها النقية أو أشكالها الصيدلية تتوافق تماما مع الطرق الدستورية والمسجلة.

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