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OXIDIZED DIPHENYLAMINE AS A SPECTROPHOTOMETRIC REAGENT FOR THE DETERMINATION OF SOME PHARMACEUTICAL THIOLS AND THIOAMIDES

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تم استحداث استخدام ثنائى فينيل أمين المؤكسد في التحليل الطيفي لتقدير ستة مركبات صيدلية هامة من مجموعة الثيول والثيوأميد. وهذه المركبات هي: أسيتيل سيستايين ، كابتوبريل ، كاربيمازول ، بروبيل ثيويوراسيل ، ثيوبنتال الصوديوم ، تيوبرونين. واعتمدت الطريقة المستخدمة على قياس النقص في كثافة اللون البنفسجي ذي الطول الموجي ٥٨٠ نم ، الخاص بمركب ثنائى فينيل أمين المؤكسد (ثنائى فينيل بنزيدين). ووجد أن هذا النقص نتيجة لتأثير العقاقير المحللة كعوامل مؤكسدة وأنه يتناسب مع تركيزاتها. واستخدمت كبريتات الحديديك في وسط من حمض الكبريتيك المركز في الأكسدة الآنية لمادة ثنائى فينيل أمين. ووجد أن النسبة المولارية هي اثنان من ثنائى فينيل أمين مع واحد من أيون الحديديك. وتمت دراسة التغيرات في ظروف التفاعل المستخدم ومنها تم تحديد نوع الحمض وتركيزه المناسب ووقت التفاعل ودرجة ثباته وكذلك أنسب المذيبات لتخفيف مخلوط المتفاعلات. وتم أيضا دراسة عناصر صلاحية الطريقة وشملت مدى التركيزات التي تحقق علاقة خطية والحد الأدنى للكشف والحد الأدنى للتقدير ومدى التكرارية ومدى الاختيارية لهذه الطريقة المقترحة. واتضح أن الطريقة بسيطة وحساسة ودقيقة. وتم تطبيقها في تقدير المركبات المدروسة في العديد من المستحضرات الصيدلية المتاحة في السوق المحلية وثبت التوافق الكبير في النتائج مع مثيلاتها من الطرق الدستورية.

Oxidized diphenylamine is newly utilized as a redox spectrophotometric reagent for the determination of six pharmaceutically important thiol and thioamide drugs named: acetylcystiene, captopril, carbimazole, propylthiouracil, thiopental sodium, and tiopronin. The method is based on measurement of the decrease in absorption intensity of the oxidized diphenylamine (diphenylbenzidine violet, λmax= 580 nm) reagent as a result of the reduction effect of the analysed drugs. This reagent was instantaneously prepared by the oxidation of diphenylamine using ferric sulphate in sulphuric acid medium. The molar ratio of the chromogen reagent was determined to be 2:1; diphenylamine : iron (III). The decrease in colour intensity was found to be quantitatively dependant on drug concentration. Experimental variables including reagent concentration, acid type and concentration, dilution solvent, reaction time, temperature and stability were studied and optimized. Validation parameters including linearity range, detection and quantitation limits, precision, selectivity and robustness were evaluated. The proposed method was found to be simple, sensitive and accurate one indicated by the studied validation parameters. Good recoveries (98.0±0.14 – 100%, ±0.98) were obtained by the suggested method and it was applied for the determination of the studied drugs in many pharmaceutical dosage forms available in the local market. Good agreement, indicated by acceptable t- & F- tests, was found between results obtained by the suggested method and those obtained by the reported or pharmacopoeial methods.

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INTRODUCTION

Thiols and thioamides represent an important class of compounds with an intensive and interesting chemistry and pharmacology. N- Acetylcysteine is a mucolytic drug. Also it has a key role in the management of paracetamol overdosage. Captopril is a thiol drug that has been used in management of hypertention, in heart failure, following myocardial infarction and in diabetic nephropathy. Carbimazole is used in the management of hyperthyrodism, the treatment of Grave's disease, the preparation of hyperthyroid patients for thyroidectomy, as an adjunct to radio-iodine therapy and treatment of thyroid storm. Propylthiouracil is used in the management of hyperthyrodism. Thiopental is an intravenous anesthetic drug. Tiopronin is used for management of cysteinuria, rheumatoid arthritis, hepatic disorders, heavy metals poisoning and as mucolytic in respiratory disorders.[1,2] The chemical structures[3] of the studied drugs are shown in Table 1.

Diphenylamine and its family are redox titrimetric indictors. In the presence of oxidants e.g. potassium dichromate, it undergoes an irreversible oxidative coupling reaction to yield diphenylbenzidine which is also colourless. However, upon further oxidation, it is converted into a bright purple compound knawn as diphenylbenzidine violet.[4] On the other hand, diphenylamine has been previously utilized to produce a chromogen which is structurally methylene blue like dye as a condensation product with oxidized phenothiazine compounds. N-bromo-succinimide was used phenothiazine oxidation and this method was applied for the colourimetric determination of many drugs in their pharmaceutical dosage forms.[5] Oxidized diphenylamine derivatives have been previously utilized and reviewed for the spectrophotometric determination of ClO_4^-, ReO_4^-, Ir(IV), Ru(IV), and Au(III).[6]

In this work, oxidized diphenylamine is newly utilized as a chromogen for the spectrophotometric determination. It has been successfully applied on the determination of six thiol and thioamide drug compounds of medicinal value.

Many analytical methods have been published for the analysis of the studied thiols and thioamids. Concerning colorimetric methods reported for the determination of the studied drugs, some selected methods are shown in Table 2.

EXPERIMENTAL

Apparatus

1. UV-1601 PC**,** UV-Visible Spectro-photometer (Shimadzu, Japan).
2. Ultrasonic cleaner (Cole-Parmer, Chicago, USA).
3. A sensitive balance (Precisa, Presisa Instruments Ltd., Switzerland).
4. MLW type, thermostatically controlled water bath (Achtung, Germany).

Materials

Diphenylamine (Merck, Darmstadt, Germany), Iron **(**III**)** sulfate (Winlab Co., UK), carbimazole (Cid Co., Talbya, Giza, Egypt), captopril (Bristol, Meyers Squibb), Acetylcysteine (Sedico, 6th of October, Cairo, Egypt), thiopental sodium (EIPICO, 10th of Ramadan, Cairo, Egypt), propylthiouracil (Amoun Pharm. Co., El-obour city, Cairo, Egypt) and tiopronin (Aldrich Co., USA), ammonium vanadate (Winlab Co., UK), ceric ammonium sulfate (Sigma Co. St. Louis, USA) and p-dimethylaminobenzaldehyde (Winlab Co., UK). All drugs were used as working standards without further purification. They were analyzed according to official or reported methods to determine their purity and compliance with the pharmaceutical requirements.

Analytical grade sulfuric acid, perchloric acid, nitric acid, hydrochloric acid, acetic acid, acetonitrile, methanol, ethanol and acetone were used throughout this study. Double distilled water was used throughout the work.

Formulations

Pharmaceutical preparations listed in Table 3 were purchased from the local market and subjected to analysis by the proposed procedure. Tiopronin tablets were laboratory prepared as synthetic mixture according to reported requirements.[37,41]

Table 1: Chemical structures[3] of the studied thiol and thioamide drugs.

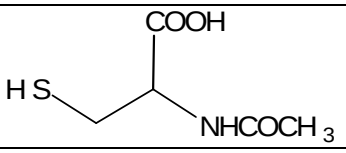
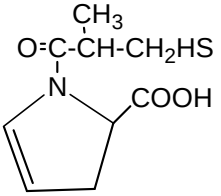
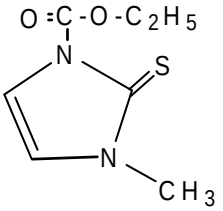
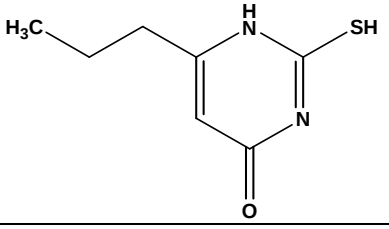
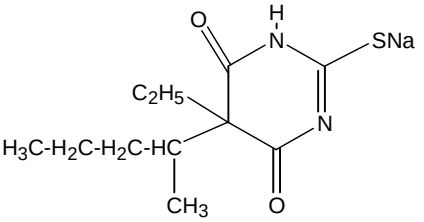
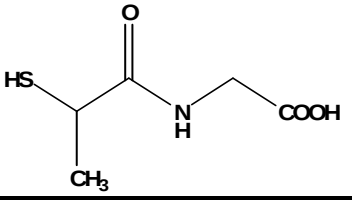
Drug	Structure	Molecular Weight
N-Acetylcysteine		163.2
Captopril		217.3
Carbimazole		186.2
Propylthiouracil		170.2
Thiopental sodium		264.3
Tiopronin		163.2

Table 2: Some selected colorimetric methods used for the determination of the studied thiol and thioamide drugs.

Drug	Colorimetric reagent	Ref.
N-Acetylcysteine	Palladium (II), and cobalt-ethylenediamine tetraacetate (EDTA).	7
	Fe (III) -ferrozine and sodium perchlorate.	8
Captopril	Citric acid, acetic anhydride	9
	Potassium bromate and celestine blue.	10
	Fe (III) and 1, 10- phenanthroline.	11
	Sodium nitrite.	12
	Folin- Ciocalteu.	13
	Ammonium molybdate and phosphoric acid in presence of Cu (II).	14
	Fe (III) and Potassium ferricyanide in sulfuric acid medium.	15
	Ellman's and N-ethylmaleimide reagents.	16
	Azure A and B.	17
	Metol and dichromate in phthalate buffer.	18
	Cu (II) and neocuproine.	19
	Tetrazolium blue.	20
	Methylbenzothiazolinone hydrazone (MBH) and diamine-2 HCl.	21
	Phosophotungestic acid.	22
	N-bromophthalimide and molybdophosphoric acid.	23
	Iodine-starch complex.	23
	Fe (III) and 2, 2 '-bipyridyl.	24
	Sodium nitrite and cresyl fast blue.	25
	7, 7, 8, 8-Tetracyanoquinodimethane (TCNQ).	26
Carbimazole	Potassium dichromate and molybdate.	27
	Potassium bromate, bromide and flourescien.	28
	Mercurochrome.	29
Propylthiouracil	Neutral red and hypophosphite.	30
Thiopental sodium	Chloranil, Chloranilic acid and dichlone.	31
	Dinitrobindone.	32
	Sodium nitroprusside in basic medium.	33
	Flouranil, 2, 4, 7-trinitro-9-fluorenone, 7, 7, 8, 8-tetracyanoquino-dimethane, chloranil, chloranilic acid and tetracyanoethylene.	34
	Sodium metaperiodate and brucine.	35
Tiopronin	5, 5 '-dithiobis- (2-nitrobenzoic acid).	36
	Tetrachloropalladate.	37
N- Acetylcysteine & tiopronin	Fe (III) and 1,10-phenanthroline.	38
Captopril & tiopronin	4-Chloro-7-nitro-benzofurazan.	39
	2, 6-Dichloroquinone-4-chlorimide (DQC).	40
	$KMnO_4$-hematoxylin.	41
Captopril & thiopental sodium	2, 3-Dichloro-1,4-naphthoquinone (Dichlone).	42
Carbimazole & thiopental sodium	Hematoxylin-chloramine T.	43
Carbimazole & propylthiouracil	Palladium (II) and pyronine G.	44

Table 3: The studied commercial and laboratory prepared pharmaceutical preparations.

Pharmaceutical Preparation	Ingredients	Nominal Content (mg)	Manufacturer
Acetylcysteine	Acetylcysteine	200/backet	Sedico, 6th of October, Cairo, Egypt
Mucomyst	Acetylcysteine	200/backet	Bristol Meyers, Squibb
Capoten	Captopril	25/tablet	Bristol Meyers, Squibb
Capotril	Captopril	50/tablet	EPICO, 10th of Ramadan, Cairo, Egypt
Capozide	Captopril + Hydrochlorothiazide	50/tablet 25/tablet	Bristol Meyers, Squibb
Carbimazole	Carbimazole	5/tablet	CID, Giza, Egypt
Thyrocil	Propylthiouracil	50/tablet	Amoun Pharm. Co., El-obour city, Cairo Egypt.
Thiopental sodium	Thiopental sodium	1000/vial	EPICO,10th of Ramadan, Egypt.
Tiopronin	Tiopronin	0.3/50g mix.	Lab. prepared mixture.

Reagents and solutions

Iron (III) sulfate solution

Aqueous solution of iron (III) sulfate ($2x10^{-3}$M) was prepared by dissolving 80 mg in 100 ml distilled water.

Diphenylamine solution

A solution of ($4x10^{-3}$ M) was prepared daily by dissolving 70 mg of diphenylamine in 100 ml concentrated sulfuric acid.

Preparation of standard solutions

An accurately weighed amount of each of the studied drugs was transferred into a 100-ml volumetric flask, dissolved in about 50 ml of acetonitrile then completed to the mark with the same solvent to provide a stock standard solution containing about 0.70 mg/ml. The working standard solutions were prepared by further dilution with the same solvent to obtain concentrations covering the range of 4-700 µg/ml. The stock and working standard solutions were kept refrigerated in light protected flasks.

Preparation of sample solutions

Tablets and laboratory prepared mixture

Twenty tablets were weighed, finely powdered and mixed thoroughly. An accurately weighed amount of the powdered tablets equivalent to about 20 mg of the drug was transferred into 100-ml volumetric flask containing about 50 ml of acetonitrile. The contents were shaken well for 15 minutes then completed to 100 ml with the same solvent. The resulting solution was filtered off rejecting the first portion of the filtrate. The prepared solution was then diluted quantitatively with the same solvent to obtain about 300 µg/ml captopril, 50 µg/ml carbimazole, 200 µg/ml propylthiouracil and 200 µg/ml tiopronin.

Backets

Content of twenty backets were accurately weighed and a quantity equivalent to 25 mg of the drug was transferred into 100 ml volumetric flask and the procedures were completed as under tablets to obtain about 250 µg/ml acetylcysteine.

Vials

The content of 10 vials were mixed thoroughly and an accurately measured amount equivalent to about 40 mg of the drug was transferred into 100-ml volumetric flask and the procedures were completed as under tablets to obtain about 40 µg/ml thiopental sodium.

Molar ratio of diphenylamine-iron (III) sulfate combination

Job's method[45] of continuous variation was employed. Equimolar ($2x10^{-3}$) solutions of each of iron (III) sulfate and diphenylamine were prepared. A series of 10 ml portions of mixtures of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (Iron(III) sulfate ($2x10^{-3}$M) : diphenylamine

($2x10^{-3}$M) were made up in different complementary proportions in 10-ml calibrated flasks. The flasks were heated in a thermostatically controlled water bath for 2-3 minutes at 60° and the absorbances of the solutions were measured at 580 nm.

General assay procedure

Into a 10-ml calibrated flask, one ml of iron (III) sulfate ($2x10^{-3}$ M) and one ml of diphenylamine ($4x10^{-3}$ M) were mixed. The contents of the flask were heated in a thermostatically controlled water bath for 2-3 minutes at 60°. One ml of the standard or the sample preparation in the range of 4.0-700.0 µg/ml was added and the solutions were heated again at 60° for 5 min. The reaction mixture was then cooled and diluted to the mark with acetonitrile. The decrease in absorbance was measured at 580 nm. against blank which was prepared similarly omitting the addition of drug sample.

RESULTS AND DISCUSSION

Spectral characteristics

Diphenylamine in the presence of iron(III) sulfate is oxidized into colorless diphenylbenzidine, which is reversibly further oxidized to diphenylbenzidine violet.[46] The studied drugs namely, acetylcysteine, captopril, carbimazole, propylthiouracil, thiopental sodium and tiopronin have reducing properties, so they will decrease the intensity of the violet colour of diphenylbenzidine (Scheme 1). The amount of this decrease measured at 580 nm was found to be dependant on the drug concentration and could be used for their determinations (Fig. 1).

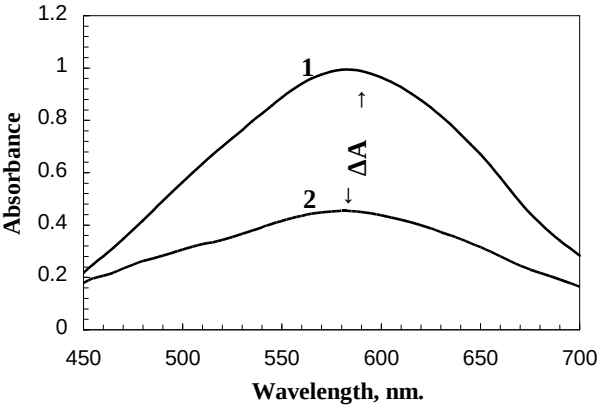
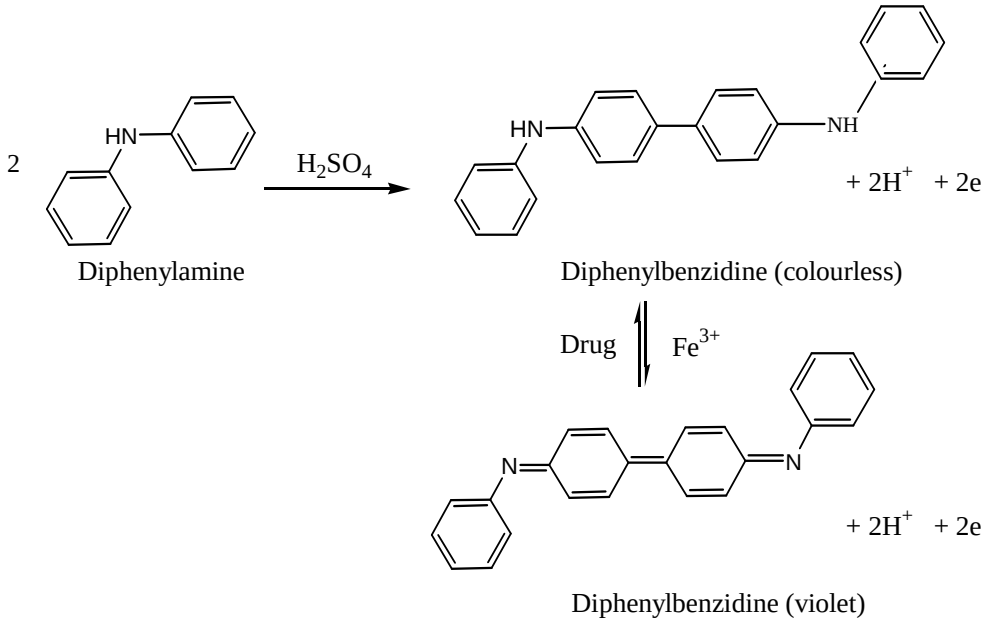


Fig. 1: Absorption spectrum of Fe (III) ($2x10^{-3}$ M)-diphenylamine ($4x10^{-3}$ M) reagent (**1**) and carbimazole (5 µg/ml) –reagent (**2**).

Molar ratio of iron (III) sulfate-diphenylamine combination

Using Job's method of continuous variation,[45] the molar ratio of iron (III) sulfate : diphenylamine was found to be 1:2 (Fig. 2). Therefore, this reagent was prepared in that ratio for subsequent work.



Scheme 1, Suggested reaction mechanism

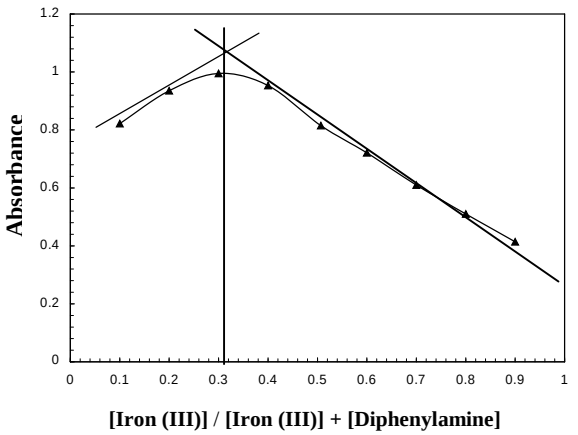


Fig. 2: Continuous variation plot obtained from solutions of iron (III) and diphenylamine ($2x10^{-3}$ M).

Optimization of variables

Various parameters affecting the reaction conditions including, reagent concentration, acid type and concentration, dilution solvents, reaction time and stability were investigated and optimized for all the studied drugs.

1- Concentration of the reagent

According to the above mentioned reaction involved, iron (III) sulfate and diphenylamine were prepared by mixing them in the ratio of 1:2. The selected concentration of this reagent which gave the highest absorption value within the practical sensitivity range of absorbance (0.9 ~1.0) was found to be ($2x10^{-3}$ M) iron (III) sulfate and ($4x10^{-3}$ M) diphenylamine.

2- Acid type and concentration

As represented in scheme 1, it is clear that the oxidation reaction of diphenylamine requires an acid medium. Therefore, different acids such as sulfuric, perchloric, nitric, hydrochloric and acetic were tested to determine the most suitable acid for the reaction. It was noticed that, perchloric acid and nitric acid, in absence of iron(III) sulfate, cause oxidation of diphenylamine indicated by the formation of the violet color of diphenylbenzidine violet. Hydrochloric acid and acetic acid did not form the violet color upon addition of iron (III) sulfate solution; may be due to the reducing properties of hydrochloric acid and the lower acidity strength of acetic acid. So, sulfuric was selected as a suitable acid for the preparation of the reagent. It was preferably added as a dissolution solvent for diphenylamine.

The effect of the concentration of sulfuric acid used for this dissolution was studied using different concentrations ranged from 4-18 M. Concentrated sulfuric acid (i.e. 18 M) developed the maximum color intensity upon addition of ferric sulfate solution. So, it was selected for this purpose.

3- Dilution solvents

Water, methanol, ethanol, acetonitril and acetone were tried for dilution of the reaction mixture. Captopril (30 µg/ml) and carbimazole (5 µg/ml) were used as a representative examples for this experiment. It was noticed that water causes fading of the produced color while, acetonitrile gave maximum absorption difference (ΔA) (Table 4). So, acetonitrile was selected as dilution solvent for subsequent work.

Table 4: Effect of different dilution solvents on absorption intensity.

Solvent	Absorption difference (ΔA)*	
	Carbimazole**	Captopril**
Acetonitrile	0.538	0.525
Methanol	0.384	0.421
Ethanol	0.345	0.389
Acetone	0.254	0.287

* Average of three determinations.
** Concentration used; 5 µg /ml carbimazole and 30 µg /ml captopril.

The effect of water used for the preparation of iron (III) sulphate reagent is compensated by the high acidity of sulphuric acid used and the presence of acetonitrile as an organic solvent present in the reaction mixture.

4- Reaction time and temperature of iron(III) sulfate–diphenylamine combination

The reaction time between iron (III) sulfate ($2x10^{-2}$ M) and diphenylamine ($4x10^{-3}$ M) was studied at three different temperature levels; 25, 60 and 100° using a thermostatically controlled water bath. It was found that at room temperature (25°), absorption intensity increases by increasing time, attaining maximum after 15 min. At 60°, the maximum intensity was attained after 2-3 min. At 100°, fading of the produced chromogen was found

to increase with heating time (Fig. 3). Therefore, the water bath was controlled at 60° for this work.

As shown in Fig. 3, the produced chromogen at 60° is almost stable for at least 30 min. Therefore, this temperature level was applied for the general assay procedure.

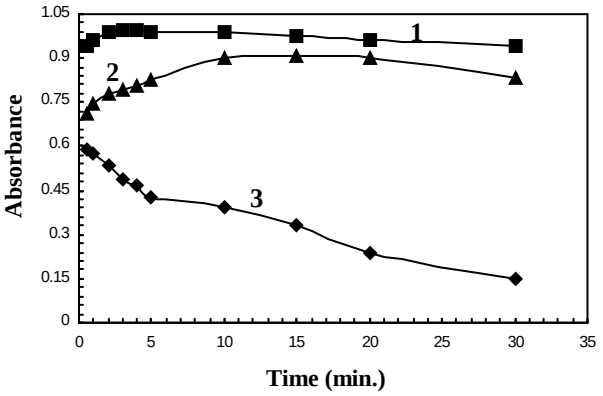
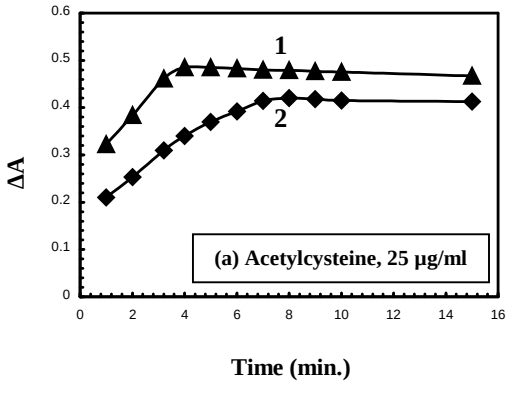


Fig. 3: developing time and stability of diphenylamine ($4x10^{-3}$M) - Iron (III) ($2x10^{-3}$M) at three temperature levels: (**1**) 60°, (**2**) 25° and (**3**) 100°.

5- Reaction time and stability of drug-oxidized diphenylamine combination

The reaction time between oxidized diphenylamine and studied drugs was studied at two temperature levels, 25 and 60°. It was found that at 25°, the absorbance difference attains its maximum after relatively longer time than that at 60°. In addition, this difference is usually higher at 60° than it is at 25° (Fig. 4; a-f). Therefore, 60° temperature level was selected and optimal reaction time of 5 min was determined for the general assay procedure. The produced chromogen intensity was found to be almost stable for at least 15 min (Fig. 4; a-f).



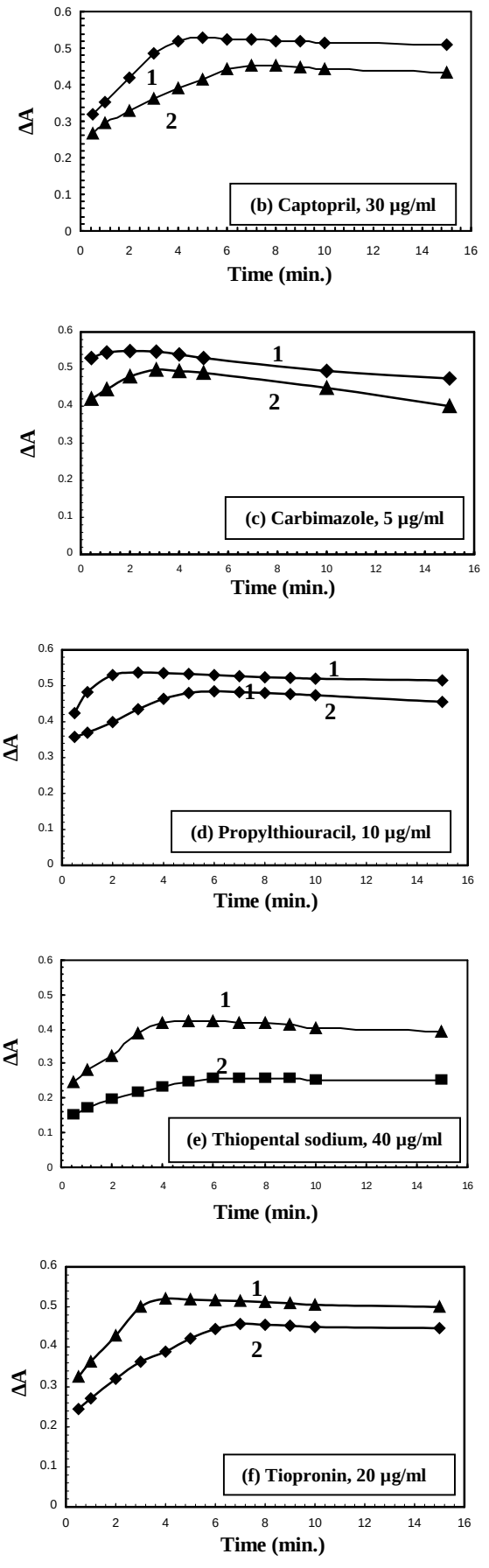


Fig. 4(a-f): Reaction time of drug-oxidized diphenylamine at two temperature levels; (**1**) 60° and (**2**) 25°.

Validation of the proposed method

1- Linearity, detection and quantitation limits

The calibration curves of the studied drugs were constructed by plotting absorbance difference versus drug concentration. Linear calibration graphs were obtained in the concentration range of final dilution cited in (Table 5). The slope (b) was used as a measure of the sensitivity of the proposed method, while the intercept (a) was used as a measure of the interfering background. Results indicate high sensitivity and low background effect of the proposed method. The correlation coefficients ranged from 0.9990–0.9998 indicating good linearity. Limit of detection (LOD) and limit of quantitation (LOQ) for the analytes were calculated according to the USP 2002[47] as follows;

$$\text{LOD or LOQ} = K.\ \sigma\ a / b$$

Where, K= 3, for LOD and K= 10, for LOQ; σ a: is the standard deviation of the intercept (a) and b: is the slope.

2- Precision

The precision of the proposed method was checked by replicate analysis of ten separate solutions of working standards of captopril and carbimazole as representative examples for the investigated drugs. The analysis was carried out at three concentration levels; 15, 30 and 45 µg/ml for captopril and 3,5 and 9 µg/ml for carbimazole. The obtained relative standard deviations were 1.302, 1.251 and 1.141% in case of captopril and 1.157, 1.297 and 1.308% in case of carbimazole. These results, being less than 2.0% indicate good repeatability.

3- Selectivity

In this study, the method was carried out for the analysis of captopril (30 µg/ml), as a representative example, in the presence of some common excipients and additives such as; starch, glucose, sucrose, gum acacia, lactose, Mg-stearate and in the presence of hydrochlorothiazide. Good recoveries were obtained. Results shown in Table 6 indicate that the proposed method is able to access the analyte in presence of common excipients, so the proposed method can be considered a selective one.

4- Robustness

The robustness of the proposed method was carried out to evaluate the influence of small variation in the reaction conditions on the method suitability and sensitivity. Using captopril (30 µg/ml) and carbimazole (5 µg/ml) as representative examples, it was found that neither small variation on diphenylamine regent concentration nor small variation on reaction time significantly affects percentage recovery of the drug (Table 7). This provides an indication of reliability the proposed method during normal use of the method in determination of the studied drugs. So, the method is considered to be robust.

Table 5: Quantitative parameters and statistical data for the analysed drugs.

Drug	ε,L.mole^{-1} cm^{-1}	Linearity range µg/ml	LOD	LOQ	Slope ± (SE)	Intercept ± (SE)	r	r^2
Acetylcysteine	3114	4-40	1.19	3.95	0.0192 (0.0003)	-0.0114 (0.0076)	0.9994	0.9988
Captopril	4007	5-45	1.35	4.49	0.0189 (0.0002)	-0.0116 (0.0085)	0.9993	0.9986
Carbimazole	20445	0.4-9	0.10	0.34	0.1115 (0.0067)	-0.0071 (0.0038)	0.9998	0.9996
Propylthiouracil	9397	2-16	0.48	1.59	0.0527 (0.0008)	0.0225 (0.0084)	0.9992	0.9985
Thiopental sodium	2901	8-70	2.31	7.69	0.0113 (0.0002)	-0.0105 (0.0087)	0.9992	0.9984
Tiopronin	4352	4-35	1.10	3.67	0.0234 (0.0004)	0.0593 (0.0086)	0.9993	0.9986

LOD: Limit of detection; µg/ml, LOQ: Limit of quantitation; µg/ml, r: Correlation coefficient.

Table 6: Analysis of captopril in the presence of some common excipients and hydrochlorothiazide.

Ingredient	Amount added (mg)	% Recovery* ± SD
1- Starch	50	98.83 ± 0.60
2- Glucose	10	100.09 ± 0.87
3- Sucrose	50	99.65 ± 0.91
4- Gum acacia	10	98.55 ± 1.04
5- lactose	10	100.06 ± 0.95
6- Mg- stearate	10	98.78 ± 0.87
7- Hydrochlorothiazide	25	98.91 ± 0.97

* Average of five determinations.

Table 7: Robustness of the oxidized diphenylamine method.

Variation	% Recovery ± SD	
	Carbimazole*	Captopril*
Oxidized diphenylamine concentration (M):		
4.2×10^{-3} M	99.12 ± 0.91	98.88 ± 0.79
3.8×10^{-3} M	99.58 ± 0.74	98.97 ± 0.87
Reaction time (min):		
5.5	98.78 ± 0.97	99.14 ± 0.98
4.5	99.25 ± 0.94	99.54 ± 0.74

* Drug concentration used; 30 µg /ml captopril and 5 µg /ml carbimazole.

Table 8: Analysis of studied drugs in their pharmaceutical preparation or laboratory prepared mixture.

Product	Ingredient (Content, mg)	%Recovery*± SD			
		Proposed method	Reference method**	F-value	t- value
1- Acetylcysteine (backets)	Acetylcysteine (200)	99.85 ± 0.53	99.5 ± 0.94	2.99	1.51
2-Mucomyst (backets)	Acetylcysteine (200)	99.13 ± 0.42	99.05 ± 0.61	2.13	1.27
3-Capoten (tablets)	Captopril (25)	99.71 ± 0.83	99.43 ± 0.75	1.22	1.17
4-Capotril (tablets)	Captopril (50)	99.82 ± 0.67	99.27 ± 0.79	1.39	1.41
5-Capozide (tablets)	Captopril (25)	99.55 ± 0.72	99.91 ± 0.33	4.63	1.07
6-Carbimazole (tablets)	Carbimazole (5)	97.98 ± 0.98	97.93 ± 0.12	1.52	0.78
7- Thyrocil (tablets)	Propylthiouracil (50)	99.18 ± 0.43	98.93 ± 0.42	1.09	1.05
8-Thiopental sodium (vial)	Thiopental sodium (1000)	99.72 ± 0.14	99.18 ± 0.72	3.65	1.61
9-Tiopronin (mixture)***	Tiopronin (100)	99.18 ± 0.43	98.93 ± 0.42	1.09	1.05

*Average of six determinations ±SD. ***Laboratory prepared mixture
**References No. 47 & 48. Theoretical values at 95% confidence limit t= 2.31 and F= 5.05

Application on pharmaceutical preparations

Some commercial dosage forms of the studied drugs were successfully analyzed by the proposed and the official USP 2002[47] and British Pharmacopoeia 1998.[48] Recovery experiments were performed for each drug in its dosage forms. Results are shown in Table 8. Dosage forms of tiopronin are not available in local market, so its analysis was carried out using laboratory prepared mixture. There is no significant difference between results obtained by proposed, official or reported methods, as indicated by t- and F- tests (Table 8).

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