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Fluorimetric determination of some thiol compounds in their dosage forms

Sh.M. Al-Ghannam *, A.M. El-Brashy, B.S. Al-Farhan

Department of Chemistry, Girl's College of Science, PO Box 838, Dammam 31113, Saudi Arabia

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Abstract

A simple fluorimetric procedure was adopted for determination of three pharmaceutical compounds containing thiol groups namely, captopril, D-penicillamine and *N*-acetylcysteine. In this method, the drugs are treated with 1,2-naphthoquinone-4-sulfonic acid. The latter is reduced to 1,2-dihydroxynaphthalene-4-sulfonic acid which has a maximum fluorescence intensity at 480/318 nm (λEm/Ex). The method is sensitive to 0.5–4.5 μg ml^{-1} with minimum detectability 0.05 μg ml^{-1} ($S/N = 2$), and has been applied to determine these three thiols in their dosage forms. The results obtained are compared favourably with those obtained by their pharmacopeial methods.

Keywords: Captopril; *N*-acetylcysteine; D-penicillamine; 1,2-Naphthoquinone-4-sulfonic acid; Dosage forms

1. Introduction

The studied compounds belong to different pharmacological classes [1], captopril is used in treatment of hypertension, penicillamine is used in treatment of Wilson's disease and rheumatoid and *N*-acetylcysteine has a mucolytic effect in respiratory diseases. Due to their great importance in chemotherapy and widespread use, several reports were published concerning their determination in dosage forms and biological fluids.

The United States Pharmacopeia-24 [2] recommended an iodometric titration for captopril and high performance liquid chromatography for *N*-acetylcysteine and Penicillamine. Other reported methods included: titrimetry [3,4], spectrophotometry [5,6], fluorometry [7–11], polarography [12,13], chromatography [14,15], chemiluminescence [16,17] and capillary electrophoresis [18,19] for determination of captopril, *N*-acetylcysteine and penicillamine in pure form, in dosage forms and in biological samples.

Some reagents have been used for fluorometric determination of the studied thiol compounds such as cerium (IV) [7], *N*-[4-(2-phthalimidyl)phenyl] maleimide [8], 4-(6-methyl-2-naphthyl)-4-oxobut-2-enoic acid [9], 4[*NN*-dimethyl-aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole [10] and 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole [11].

The aim of this research is to apply a simple, accurate and precise fluorimetric procedure for determination of three pharmaceutically important drugs: captopril, *N*-acetylcysteine and penicillamine in their dosage forms.

2. Experimental

2.1. Apparatus

An Amino-Bowman Model J_4-9860 spectrofluorometer was used with the excitation and emission slits set at 5 mm. A 1-cm quartz cell was used for all measurement, [λ emission at 480 nm and λ excitation at 318 nm].

2.2. Materials

D-penicillamine was obtained from Bayer, Germany, *N*-acetylcysteine from Pharzam, Grafeling, Germany and captopril from Squibb, N.I. USA. The purity of these drugs was established by applying the recommended official methods [2].

* Corresponding author.
E-mail address: sn_ghannam@yahoo.com (S.M. Al-Ghannam).

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2.2.1. Stock solutions

Solutions (0.1%) of the studied drugs were prepared in distilled water. Diluted solutions were obtained using distilled water.

2.3. Reagents

1,2-Naphthoquinone-4-sulfonic acid (NOSA) was obtained from Aldrich Chemical Co. A 0.02% NOSA stock solution in 50% aqueous methanol was prepared every 2 days and kept in refrigerator as recommended [20].

Phosphate buffers were all prepared using disodium phosphate (0.2 M) and adjusted to the desired pH (5–8) with concentrated phosphoric acid. Each buffer solution contained 2% (V/V) *n*-butanol, added as preservative.

Distilled water was used to prepare all solutions.

2.4. Procedure

2.4.1. Preparation of calibration graphs

Aliquots containing 5–45 μg of the thiol compounds were transferred into a series of 10 ml standard flasks. NOSA (1 ml) solution and 5 ml of phosphate buffer (pH 6) were added to each flask, the volume was completed to 10 ml with distilled water and the fluorescent intensity was measured at 480 nm (emission) and 318 nm (excitation) after 15 min at room temperature. A blank reagent was prepared simultaneously. Calibration graphs were constructed by plotting the values of the percent relative intensities (%RI) against the final concentration (μg ml^{-1}) of the thiol compounds. Alternatively, the regression equations are derived.

2.4.2. Procedure for dosage forms

Weigh and pulverize 20 tablets or empty the contents of 20 capsules or five packets containing captopril, or penicillamine or *N*-acetylcysteine, respectively. Transfer an accurately weighed amount equivalent to 50 mg drug to 100 ml standard flask, add 50 ml distilled water, shake thoroughly and dilute the mixture with distilled water, filter if necessary. Analyse an aliquot of this solution according to the procedure cited before. All subsequent samples were buffered at pH 6 and allowed to stand for about 15 min at room temperature, before measurement.

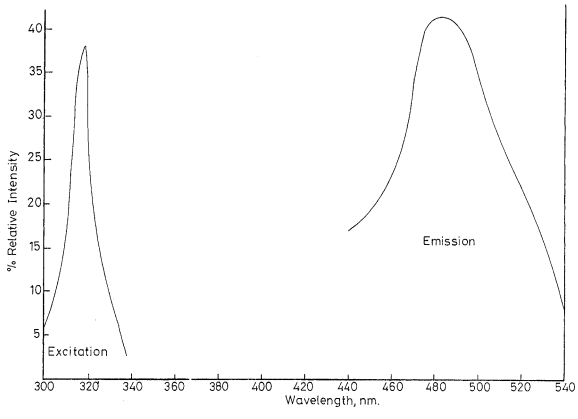
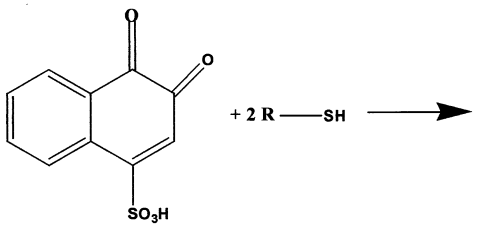


Fig. 1. Fluorimetric spectra of captopril (2 μg ml^{-1}) by 1,2-naphthoquinone-4-sulfonic acid.

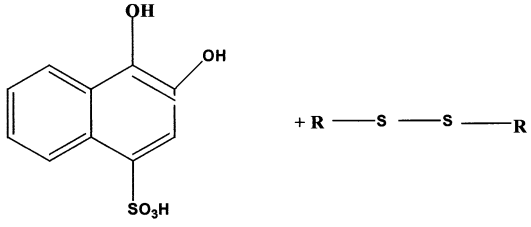
3. Results and discussion

The reaction between thiol compounds and 1,2-naphthoquinone-4-sulfonic acid depends on the reduction effect of the thiol group forming 1,2-dihydroxynaphthalene-4-sulfonic acid which has a highly fluorescence emits at 480 nm after excitation at 318 nm (Fig. 1).

The reduction of 1,2-naphthoquinone 4-sulfonate was previously discussed for determination of hydrazino compounds [20] and hydralazine drug [21]. We have found this reaction is also useful for determination of captopril, *N*-acetylcysteine and penicillamine which contain a reducing thiol group. The mechanism of the reaction will be:



1,2-Naphthoquinone 4- sulphonate



1,2-Dihydroxynaphthalene 4-sulphonate

3.1. Study of the experimental conditions

The factors affecting the reaction of thiol compounds and NOSA were first studied. All factors were kept constant except one, which was changed in turn to study its effect.

3.1.1. Effect of pH

The reduction effect of the thiol compounds to NOSA depends on pH. The effect of pH was studied and indicated in Fig. 2. From this Figure, as the pH of

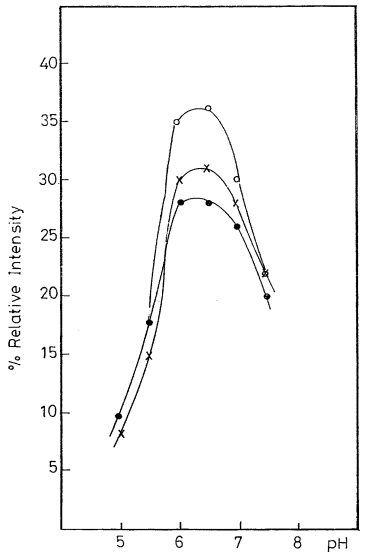


Fig. 2. Effect of buffer on the reaction between thiol compounds and 1,2-naphthoquinone-4-sulfonic acid. o——o Captopril (2 μg ml^{-1}). •——• Penicillamine (2 μg ml^{-1}).x——x *N*-acetylcysteine (2 μg ml^{-1}).

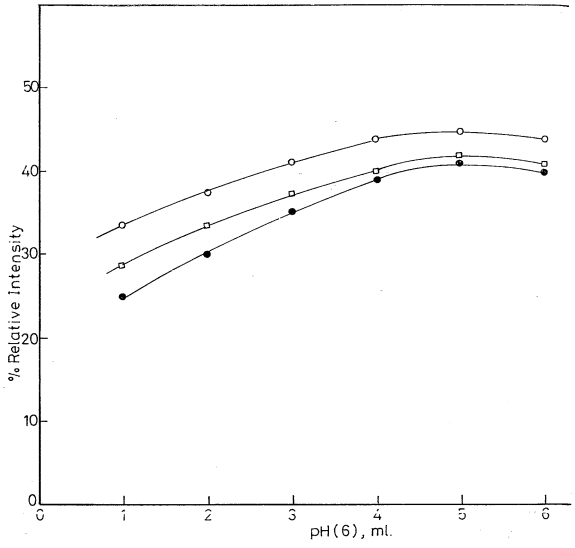


Fig. 3. Effect of buffer (pH 6) concentration on the reaction between thiol compounds and 1,2-naphthoquinone-4-sulfonic acid.o——oCaptopril (2 μg ml^{-1}) □——□ Penicillamine (2 μg ml^{-1}).•——•*N*-acetylcysteine (2 μg ml^{-1}).

the reaction increased, the fluorescent intensity increased until reached pH 6–6.5, after that it decreased. Fig. 3 shows that 5 ml of pH 6 was sufficient for maximum intensity.

3.1.2. Effect of reaction time

The reaction of the studied thiol compounds and NOSA depends on time. Maximum fluorescent intensity was observed after 15 min, at room temperature and after 30 min, the fluorescence decreased (Fig. 4). This period (30 min) was sufficient to perform all measurements.

3.1.3. Effect of NOSA concentration

The effect of NOSA was studied by using increasing volumes of 0.02% NOSA in aqueous methanol (50%). It was found that 1 ml of the reagent is appropriate for maximum fluorescence intensity. Excess volumes of the reagent, had little effect on fluorescence, as shown in Fig. 5.

After optimizing the conditions, it was found that the relation between the %R.I and final concentrations of the thiol compounds was rectilinear in the range 0.5–4.5 μg ml^{-1} with a minimum detectability ($S/N = 2$) of 0.05 μg ml^{-1}. The linear regression analysis [22] of the results are given in Table 1.

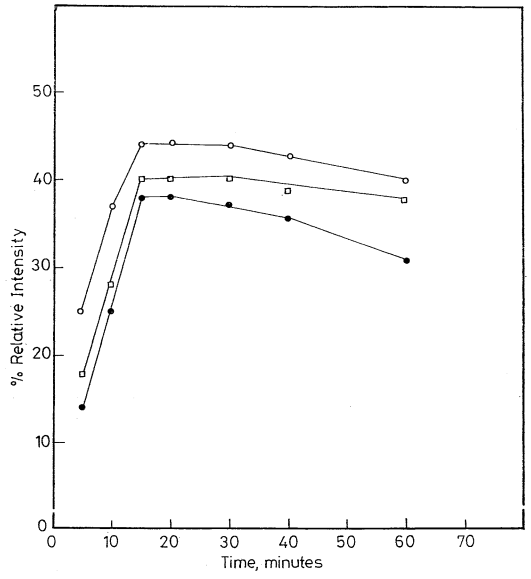


Fig. 4. Effect of time on the reaction between thiol compounds and 1,2-naphthoquinone-4-sulfonic acid. o——o Captopril (2 μg ml^{-1}). □——□ Penicillamine (2 μg ml^{-1}).•——•*N*-acetylcysteine (2 μg ml^{-1}).

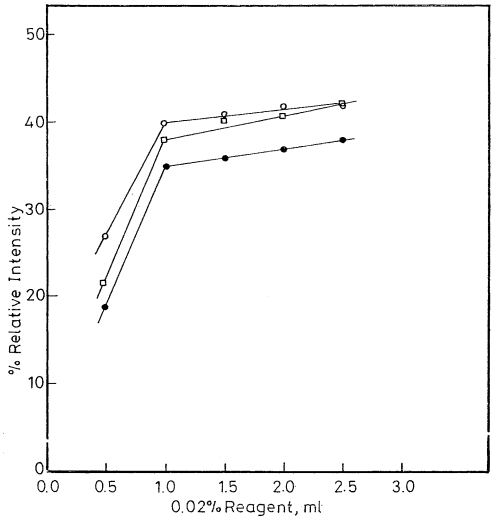


Fig. 5. Effect of 1,2-naphthoquinone-4-sulfonic acid concentration on thiol compounds.o——oCaptopril (2 μg ml^{-1})□——□Penicillamine (2 μg ml^{-1}).•——•*N*-acetylcysteine (2 μg ml^{-1}).

Table 1
Regression equations of the studied thiol compounds and 1,2-naphthoquinone-4-sulfonic acid

Comp.	Intercept	Slope	Correlation coefficient
Captopril	0.533	123.642	0.99987
Penicillamine	−0.236	185.427	0.99995
N-Acetylcysteine	0.284	164.314	0.99968

Tables 2 and 3 show the recoveries of different concentrations of the three thiol compounds alone and in their dosage forms, compared with those obtained using the pharmacopeial methods [2].

Statistical analysis of the results of authentic samples of thiol compounds obtained by both the proposed and official methods [2] revealed no significant differences between the two methods, regarding the accuracy and precision, as indicated by the *F*-test and Student's *t*-test, respectively [22] (Table 2).

Other reducing drugs were studied such as ascorbic acid, cysteine and hydrochlorothiazide which may incorporated with the studied drugs. This study reveals that, ascorbic acid, cysteine and hydrochlorothiazide need heating in water bath for at least 10 min, to give maximum intensities. So, these interferences, if present, have no effect on the estimation of the studied drugs performed by the cited conditions.

In conclusion, a simple and accurate method was developed for the determination of captopril, *N*-acetylcysteine and penicillamine in pure state and in their pharmaceutical dosage forms. Starch, glucose, maltose, magnesium stearate and other additives which incorporated in dosage forms as excipients have no effect.

Table 2
Determination of thiol compounds by 1,2-naphthoquinone-4-sulfonic acid

Comp.	Proposed			Official method [2]	
	Taken (μg ml^{-1})	Found (μg ml^{-1})	Rec. (%) [a]	Taken (mg)	Rec. (%) [a]
Captopril	0.5	0.4991	99.82	80	100.21
	1.0	0.9954	99.54	120	99.98
	1.5	1.5047	100.31	150	100.75
	2.0	2.0124	100.62	180	101.08
	3.0	3.0144	100.48		
	4.0	3.9828	99.57		
Mean ± C.V.			100.06 ± 0.47		100.51 ± 0.50
F			1.14(5.41)		
t			1.45(2.37)		
Penicillamine	0.5	0.4944	98.88	80	99.67 [b]
	1.0	0.9984	99.84	100	100.34
	1.5	1.5044	100.29	120	101.07
	2.0	2.0192	100.96	150	100.61
	3.0	3.0015	100.05		
	4.0	4.0408	101.02		
	4.5	4.5023	100.05		
Mean ± C.V.			100.16 ± 0.73		100.42 ± 0.58
F			1.58 (8.94)		
t			0.61 (2.31)		
N-acetylcysteine	0.5	0.5016	100.32	100	101.42[b]
	1.0	1.0046	100.46	120	101.10
	1.5	1.4978	99.86	150	99.72
	2.0	2.0262	101.31	180	100.07
	3.0	3.0024	100.08		
	4.0	3.9968	99.92		
	4.5	4.5185	100.41		
Mean ± C.V.			100.34 ± 0.49		100.58 ± 0.81
F			2.75 (4.76)		
t			0.62 (2.31)		

F: variance ratio, *t*: the Student's *t*-test. The values between brackets are the tabulated at $P = 0.05$ [22]. C.V.: coefficient of variation.
[a] Average of three determinations.
[b] As the titrimetric method cited for captopril [2].

Table 3
Determination of thiol compounds in their dosage forms by the fluorimetric and official methods

Dosage form	Proposed method			Official method [2]	
	Taken (μg)	Found (μg)	Rec. (%) [a]	Taken (mg)	Rec. (%) [a]
Capoten tablets (a)	1	1.0106	101.06	80	99.98
(Captopril, 25 mg per tablet)	2	2.0096	100.48	100	100.75
	3	3.0468	101.56	120	101.93
Mean ± C.V.			101.03 ± 0.54		100.89 ± 0.97
Artamine capsules (b)	1	1.0096	100.96	80	100.34
(Penicillamine, 250 mg per capsule)	2	1.9908	99.54	100	101.07
	3	2.9796	99.32	120	101.38
Mean ± C.V.			99.94 ± 0.89		100.93 ± 0.53
Acetylcysteine packets (c)	1	1.0131	101.31	80	101.10
(Acetylcysteine, 100 mg per pack)	2	1.9984	99.92	100	100.84
	3	3.0138	100.46	120	100.72
Mean ± C.V.			100.56 ± 0.70		100.89 ± 0.19

(a) Squibb & Sons Inc., New York. (b) Bayer, Germany. (c) GDS Pharma Dreieich, Germany.
[a] Average of three determinations.

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