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Derivatization with 4-Chloro-7-nitro-2,1,3-benzoxadiazole for the Spectrophotometric and Differential Pulse Polarographic Determination of Acetylcysteine and Captopril

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Abstract

Sensitive methods were developed for the determination of two sulfhydryl-containing drugs namely acetylcysteine (I) and captopril (II). The methods were based on reacting the drugs with 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) in the presence of sodium tetraborate in absolute methanol. The yellow colored products obtained were measured spectrophotometrically at 417 and 420 nm for (I) and (II), respectively and by differential pulse polarography at -872 and -1007 mV (vs. Ag/AgCl electrode) for compounds (I) and (II) after getting rid of excess unused reagent by extraction with ether. The different experimental parameters were studied and optimized spectrophotometrically. The proposed methods were validated and applied to the determination of the cited drugs in their pharmaceutical preparations. The results were statistically analyzed and compared to those of a reference HPLC method.

Key words

Spectrophotometry • Differential pulse polarography • 4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl)

Introduction

Acetylcysteine, (*N*-acetyl-L-cysteine), is a mucolytic agent used in respiratory disorders associated with active cough [1]. It has been determined in pharmaceutical preparations by several methods including spectrophotometry[2, 3],

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spectrofluorimetry after reacting with different derivatization agents[4, 5], HPLC [6, 7], capillary electrophoresis [8] , and several voltammetric techniques [9, 10].

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline, is a sulfhydryl-containing inhibitor of angiotensin converting enzyme. It is used in the management of hypertension, heart failure following myocardial infarction, and diabetic nephropathy [1]. A full bibliography of captopril up to 1982 is found in the analytical profile [11]. The spectrophotometric methods used for the determination of this drug are based on the reactivity of its tertiary nitrogen [12], mercapto group [13, 14] or complex formation [15, 16]. Captopril has also been assayed spectrofluorimetrically after reacting with fluorogenic reagents [2] or reducing Ce (IV) to fluorescent Ce (III) [17]. Other techniques adopted for the determination of captopril include voltammetry [18, 19], GC [20], HPLC [21, 22], atomic absorption spectrophotometry [23], and capillary electrophoresis [24].

4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) is an activated halide that has been used for the colorimetric determination of some primary and secondary amines [25, 26], for the fluorimetric assay of some amines and amino acids [27, 28] and also for pre-column derivatization of non responding compounds such as amino acids to enable their fluorescence or UV detection in liquid chromatography [28]. This work describes a simple method involving the reaction of acetylcysteine and captopril with (NBD-Cl) in methanolic aqueous borate buffer to form colored and polarographically active products that were measured both colorimetrically and by differential pulse polarography at a dropping mercury electrode.

Experimental

I. Apparatus

Measurements were performed using a Perkin-Elmer, lambda EZ 201 (Version 1.0) UV/VIS spectrophotometer equipped with 10 nm matched quartz cells and connected to a Panasonic Quiet KX-P 3626 printer. The spectral band width was 2.0 nm and the wavelength scanning speed was 200 nm/min. A Schott-Gerate pH meter Model CG 710 calibrated with standard buffers was used for adjusting the pH

values of the buffer solution used. A Metrohm 693 VA Processor with a Model 694 VA Stand assembly containing a multimode working electrode, a Pt rod as auxiliary electrode and a reference Ag/AgCl/3M KCl electrode was used for polarographic measurements. The dropping mercury electrode was used as working electrode. HPLC Shimadzu Model C-R7A Plus Chromatopac equipped with a UV detector SPD-10A was employed for chromatographic measurements.

II. Reagents and Materials

Authentic samples of acetylcysteine and captopril were obtained from SEDICO (Egypt) and Pharco Pharmaceuticals (Alex., Egypt), respectively. The commercial preparations were bought from the local market.

- Borate buffer was prepared as 0.05 M sodium tetraborate solution [29] and pH was adjusted with 0.1 M sodium hydroxide or 0.1 M boric acid.
- Britton-Robinson buffer [29] of pH 5 was prepared.
- 4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) 98% (ACROS Organics, New Jersey, USA) was prepared as 0.1% (w/v) solution in methanol. The reagent was stable for two weeks if kept under refrigeration.
- All chemicals and reagents used were of pure analytical grade.

Preparation of standard drug solutions

- Standard solutions of compounds (I) and (II) were prepared as 0.2 and 0.24 mg ml^{-1}, respectively, in methanol.

III. General procedure

Into different sets of 10 ml volumetric flasks, accurate volumes of standard drug solutions (Tab. 1.) were transferred. The specified volume of borate buffer pH 9 was added, followed by the appropriate volume of (NBD-Cl) methanolic solution (Tab. 1.). Solutions of (I) were heated into a thermostated waterbath at 40 ^{o}C for 15 min., then left to cool, while solutions of (II) were kept at room temperature.

For spectrophotometric measurement

Solutions of (I) were acidified with 0.1 ml of 5 M hydrochloric acid, then completed to the mark with methanol while solutions of (II) were made up to volume

with methanol without acidification. The absorbances were measured at the wavelengths of maximum absorption (Tab. 1.) against similarly treated blank.

For polarographic measurement

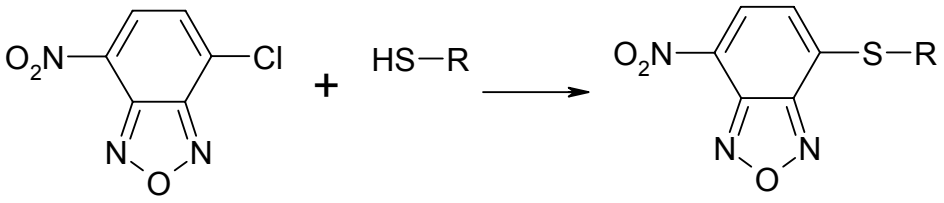
The volumes were adjusted to the mark with borate buffer pH 9 without acidification for both compounds. The solutions were extracted thrice with ether (15 ml each time). The etherial extracts were discarded and the aqueous solutions were heated at 40 ^{o}C. Aliquots from the aqueous solutions (2 ml for acetylcysteine and 1 ml for captopril) were diluted into 10 ml volumetric flasks with Britton-Robinson buffer pH 5, then the contents of the flasks were transferred into the polarographic cell and purged with pure nitrogen for 5 min. The differential pulse polarographic measurement was performed with a -100 mV pulse amplitude. The polarograms were recorded from -500 to -1100 mV vs. Ag/AgCl reference electrode at a scan rate of 20 mVs^{-1}.

IV. Procedure for dosage forms for effervescent powder of (I) and tablets of (II)

Twenty tablets or the contents of at least five sachets of (I) were weighed, powdered and mixed well. An accurately weighed amount of the powder equivalent to 20 mg of (I) and 24 mg of (II) was quantitatively transferred into 100 ml volumetric flask using methanol. The flasks were sonicated for 30 min. and the volumes were adjusted to the mark with methanol. The solutions were filtered into dry flasks and the procedure was completed as previously described under the general procedure.

Results and Discussion

Acetylcysteine and captopril are two weakly absorbing compounds that lack any significant absorbance in the UV range. Derivatization of these compounds with (NBD-Cl) in borate buffer proceeds via their sulfhydryl groups as shown in the following scheme:



NBD-Cl

Sch. 1.

The produced derivatives were measured both spectrophotometrically and polarographically due to the introduction of the electro-active chromophoric nitro group to the original drug molecules. It is most probable that the nitro group is the only functional group that undergoes reduction at the applied potential since the azomethene group is included in a conjugated system and therefore is considered as a poor candidate for reduction. The yellow colored products obtained show well defined absorption curves and differential pulse cathodic peaks as evident in Figs. 1 and 2.

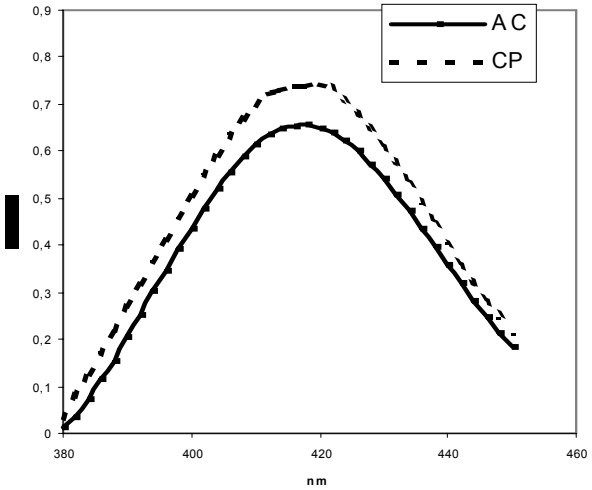


Fig. 1. Absorption curves of the NBD derivatives of acetylcysteine (8 μg ml $^{-1}$) and captopril (14.3 μg ml $^{-1}$)

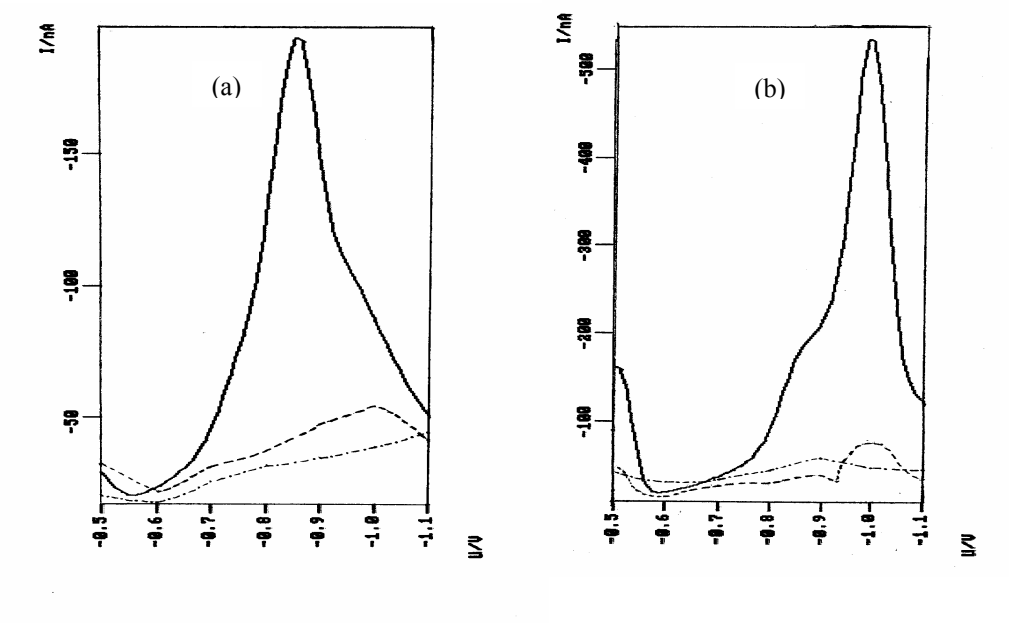


Fig. 2. Differential pulse polarograms of (a) acetylcysteine (1.4 µg ml^{-1}) and (b)captopril (1.68 µg ml^{-1}) before(-.-.-.-.-.-) and after(——) reaction with NBD-Cl against a similarly treated blank(-------) in BR buffer pH 5

I. Reaction conditions

The reaction conditions were optimized spectrophotometrically.

Optimal results were attained when 0.6 and 0.2 ml of borate buffer of pH 9, in addition to 0.5 and 0.3 ml of 0.1% w/v NBD-Cl were used for (I) and (II), respectively (Figs 3-5).

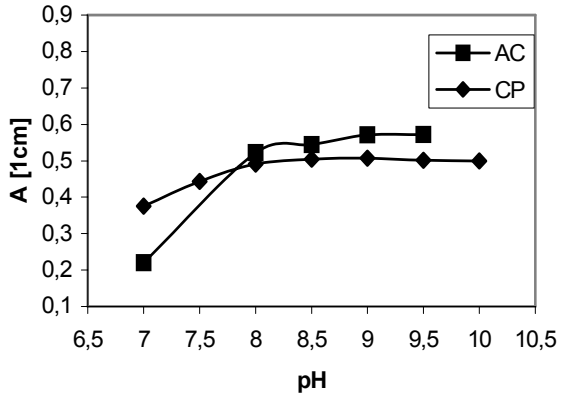


Fig. 3. Effect of buffer pH on the substitution reaction of acetylcysteine (7.0 µg ml^{-1}) and captopril (9.77 µg ml^{-1}) with NBD-Cl

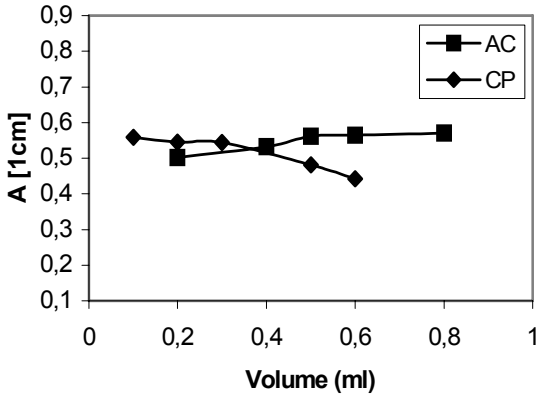


Fig. 4. Effect of borate buffer volume on the substitution reaction of acetylcysteine (6.93 µg ml $^{-1}$) and captopril (10.51 µg ml $^{-1}$) with NBD-Cl

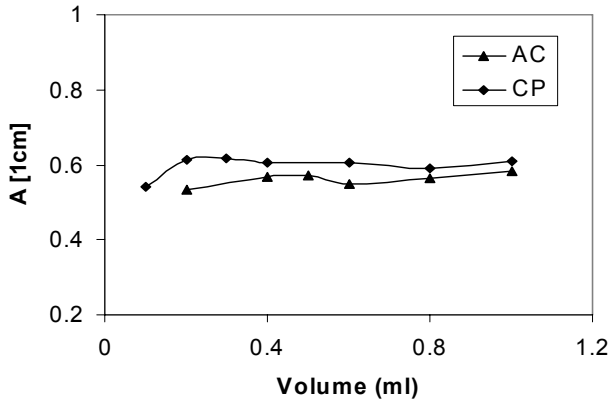


Fig. 5. Effect of NBD-Cl volume on its substitution reaction with acetylcysteine (7.0 µg ml $^{-1}$) and captopril (11.93 µg ml $^{-1}$)

The effect of reaction time and temperature was studied as it was found that (I) required heating at 40 ^{o}C for 15 min. to give full color intensity whereas for compound (II) the reaction proceeds instantaneously at room temperature just upon the addition of the reagent, moreover, the sensitivity decreases by increasing temperature (Figs 6,7).

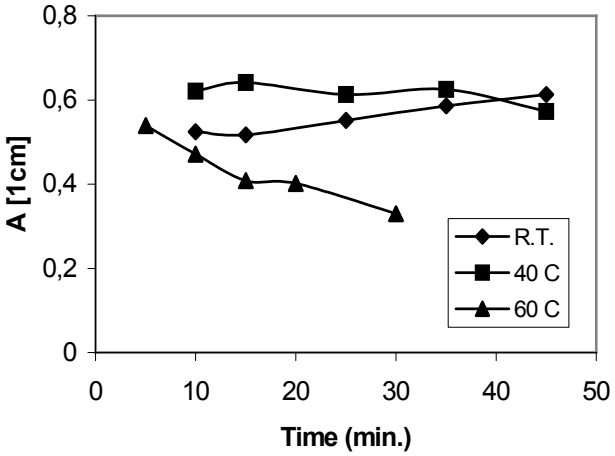


Fig. 6. Effect of heating temperature and time on the substitution reaction of acetylcysteine (7.86 µg ml $^{-1}$) with NBD-Cl

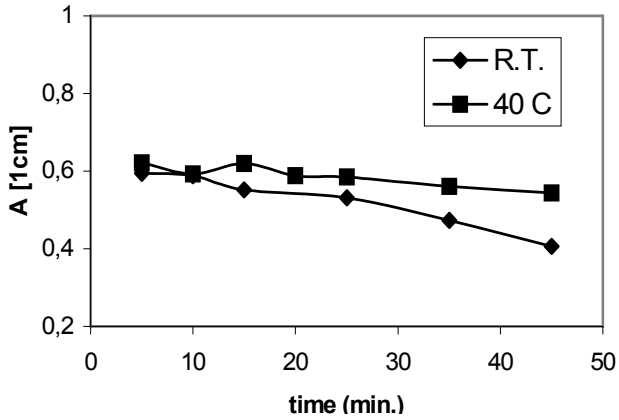


Fig. 7. Effect of heating temperature and time on the substitution reaction of Captopril (11.48 µg ml $^{-1}$) with NBD-Cl

The reaction products were stable for at least 30 min.

The spectrophotometric measurement

It has been reported [26] that heating NBD-Cl in aqueous methanolic borate buffer resulted in the formation of NBD-OCH_3 which has maximum at 372 nm and its hydrolysis product NBD-OH with a maximum at 462 nm. Addition of HCl to NBD-OH caused hypsochromic shift from 462 to 387 nm, therefore acidification of the solutions before the measurements was necessary to eliminate any blank interference between 420 and 500 nm. Compound (I) was heated at 40 ^{o}C for 15 min. and thus required this acidification step, whereas compound (II) reacted

instantaneously at room temperature with the reagent and therefore the acidification step was omitted.

The differential pulse polarographic measurement

The excess unused reagent was eliminated prior to the polarographic measurement by extraction with ether [30] The effect of Britton-Robinson buffer pH on the peak current was investigated where it was found that for both drugs maximum peak current was attained at pH 5 (Fig. 8.).

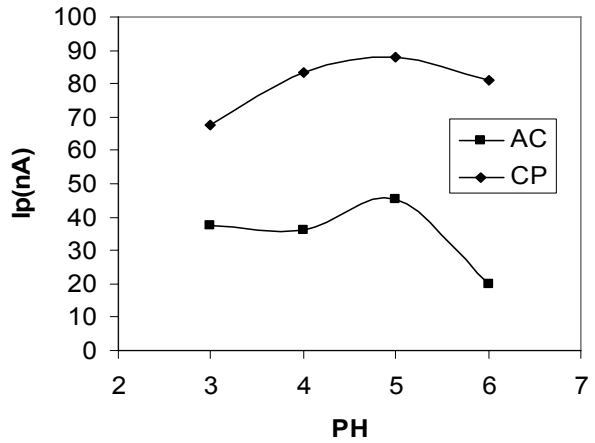


Fig. 8. Effect of Britton Robinson buffer pH on the peak current resulting from the reduction of the NBD derivatives of acetylcysteine(9.6 µg ml $^{-1}$) and captopril (9.6 µg ml $^{-1}$)

II. Validation of the proposed method

Linearity

Under the studied experimental conditions, the absorbance and the differential pulse peak current values for the spectrophotometric and the polarographic measurements, respectively, were found to be proportional to drug concentrations over the ranges stated in Tab. 1. The good linearity was manifested by the values of the variances around the slopes (S^2_b) and correlation coefficients (r).

Tab. 1. Assay parameters for the determination of acetylcysteine and captopril by the proposed reaction with NBD-Cl

Item	Drug	
	(AC)	**(CP)**
Standard conc. (mg ml^{-1})	0.2	0.24
Volume of standard solution	0.1-0.4	0.1-0.7
Borate buffer pH	9	9
Borate buffer volume (ml)	0.6	0.2
Reagent conc. (g%)	0.1	0.1
Reagent volume (ml)	0.5	0.3
Heating temperature (^oC)	45	Room temperature
Heating time (min)	15	instantaneously
5 M HCl volume (ml)	0.1	-
λ_{max} (nm)	417	420

Accuracy

The accuracy of the proposed procedures was assessed by calculating the recovery of the drugs spiked in common tablet excipients. The results are presented in Tab. 2.

Precision

The precision of the method was appraised by calculating the relative standard deviation of the assay results of three different drug concentrations, each in three replicates. The values presented in Tab. 2 are quite satisfactory.

Limit of detection and limit of quantitation

Tab. 2 shows the values of the limits of detection and quantitation for each drug by the proposed procedures.

Robustness

The robustness of the proposed procedures was demonstrated by the versatility of the experimental factors that affect the absorbance and peak current values.

Tab. 2. Validation data for the determination of acetylcysteine and captopril by the proposed reaction with NBD-Cl

Item	**AC**		**CP**	
	Spectro.	**DPP.**	**Spectro.**	**DPP.**
λ_{max} (nm) or E_p (mV)	417	-872	420	-1007
Concentration range (μg ml^{-1})	2-8	0.4-1.4	2.4-16.8	0.24-1.68
Regression equation				
Intercept (a)	$-0.4x10^{-2}$	1.18	$3.8x10^{-3}$	-20.90
Variance of intercept (S_a^2)	$4.2x10^{-5}$	1.37	$4.3\ x10^{-5}$	3.11
Slope (b)	$8.2\ x10^{-2}$	112.99	$5.1\ x10^{-2}$	294.16
Variance around slope (S_b^2)	$1.4\ x10^{-6}$	1.66	$5.4\ x10^{-7}$	3.07
Correlation coefficient (r)	0.9994	0.9998	0.9997	0.9999
Variance ($S_{y.x}^2$)	$4.0\ x10^{-5}$		$6.6\ x10^{-5}$	
Accuracy (Mean ± SD)	98.70±0.60	99.97±1.12	99.55±1.33	99.81±0.77
Precision (RSD %)	1.08	1.12	0.68	0.77
LOD (μg ml^{-1})	0.22	0.054	0.03	0.024
LOQ (μg ml^{-1})	0.74	0.180	0.11	0.081

III. Analytical applications

The results obtained by applying the proposed method to commercially available pharmaceutical preparations are presented in Tab. 3. Comparison of the

experimental data with those obtained by applying the BP 2003 [31] HPLC method of captopril to both drugs shows a relatively good correlation (Tab. 3).

Tab. 3. Assay results of acetylcysteine and captopril in their pharmaceutical preparations by the proposed methods

Item	Spectrophotometry	Reference method	DPP
Acetylcistein instant effervescent powder[a] (200 mg per sachet)			
Recovery (%) *	102.42	102.85	101.70
S.D.	0.53	0.64	1.27
T		1.16	1.81
F		1.46	3.94
Capoten™ tablets[b] (25 or 50 mg captopril per tablet)			
Recovery (%) *	101.75	101.99	100.94
S.D.	1.25	0.54	1.29
T		0.39	1.68
F		5.36	1.07

[a] Product of Sedico Pharmaceutical Co., 6 October City, Egypt.
[b] Product of Bristol-Myers Squibb Egypt.
* Each value is the mean of five measurements.
Theoretical values for t and F at P = 0.05 are 2.31 and 6.39 respectively.

Conclusions

Derivatization with NBD-Cl seems to offer several advantages as this method combines simplicity of the procedure with high sensitivity (especially for the differential pulse polarographic measurement) and also specificity as the reaction is specific to unoxidized acetylcysteine and captopril which upon oxidation are converted to the corresponding disulphides. Another added advantage is the possibility of analyzing weakly UV absorbing and/or polarographically inactive compounds through the introduction of the electroactive and spectrophotometrically active nitro group to their molecules.

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