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The Use of an Aromatic Substitution Reaction in the Spectrophotometric Determination of Selected Amino or Thiol Containing Drugs

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ABSTRACT

A simple spectrophotometric method is described for the determination of acetylcysteine (I) and captopril (II) as representative examples of thiols , as well as amlodipine besylate (III) and heptaminol hydrochloride (IV) as amine examples. The method is based on the reaction of these drugs with the activated halide 2,4-dinitrofluorobenzene (DNFB) in aqueous borate buffer to yield yellow colored products. Beer's law was obeyed over the concentration ranges of 4-12, 2.4-16.8, 8-28 and 5-25 μg mL^{-1} for (I), (II), (III), and (IV), respectively. The different experimental parameters were studied and optimized. The proposed method was validated according to the USP 27 criteria and was found suitable for the quantitation of the cited compounds in their pharmaceutical preparations without interference from common excipients.

Key words: acetylcysteine, captopril, amlodipine besylate, heptaminol hydrochloride, spectrophotometry, dinitrofluorobenzene, dosage forms

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

Captopril, 1-[(2s)-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].

Amlodipine besylate, 3-ethyl-5-methyl-2(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-pyridine-3,5-dicarboxylate monobenzenesulphonate, is a dihydropyridine calcium-channel blocker used in the management of hypertension and angina pectoris[1]. Few spectrophotometric methods have been reported for the determination of amlodipine besylate in its pharmaceutical dosage forms. These include: ion association complex formation with bromothymol blue[25] and orange (II)[26], oxidative coupling with MBTH[25], charge transfer complex formation with p-chloranilic acid[27] and difference spectrophotometry from acidic to alkaline solutions[28]. The drug has also been determined in its tablets by HPLC[29], HPTLC[30] and adsorptive square-wave anodic stripping voltammetry[31].

Heptaminol hydrochloride, 6-amino-2-methylheptan-2-ol hydrochloride, is a cardiac stimulant and vasodilator for the treatment of cardiovascular disorders[1]. Hantzch reaction followed by spectrophotometric and spectrofluorimetric measurement of the absorbance and fluorescence of the product has been a useful tool for the determination of heptaminol in its pharmaceutical preparations[32]. Other reported methods include HPLC after pre-column derivatization and either UV[33,34] or electrochemical detection[35].

The purpose of the present work was to develop a simple, rapid, accurate and sensitive method for the determination of the selected drugs in their pharmaceutical preparations by reacting them with 2,4-dinitrofluorobenzene (DNFB) in borate buffer through nucleophilic

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aromatic substitution. The results obtained were found to be in good agreement with reference methods.

MATERIALS AND METHODS

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.

II. *Reagents and Materials*

All of the chemicals and the reagents used were of pure analytical grade.

Borate buffer was prepared as 0.05 M sodium tetraborate solution[36] and the pH was adjusted with 0.1M sodium hydroxide or 0.1M boric acid.

2,4-Dinitrofluorobenzene reagent (Hopkin and Williams Co., Essex-UK) was prepared as a 0.5% W/V solution in methanol for compounds (I), (III) and (IV) and as a 0.2% W/V solution for compound (II). These solutions were freshly prepared and protected from light.

Stock solutions of compounds (I), (II), (III) and (IV) were prepared as 0.2, 0.24, 0.4 and 0.5 mg mL^{-1} in methanol, respectively. The solution of compound (III) should be protected from light.

III. *General Procedure*

Aliquots from the stock solution of each drug (Table 1) were transferred into series of 10-mL volumetric flasks. The specified volume of borate buffer of the optimum pH was added to each flask, followed by the appropriate volume of DNFB solution (Table 1). The flasks were heated on a water bath (the temperature and time of heating are cited in Table 1) and then cooled to room temperature. The volumes were made up to the mark with methanol and the absorbances were measured at the corresponding λ_{max} (Table 1) against a reagent blank treated similarly after neutralization of the solutions with 5 M hydrochloric acid.

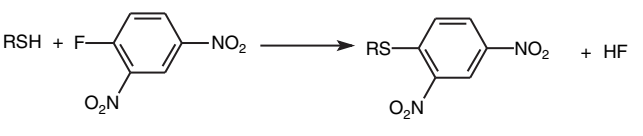
IV. *Procedure for Dosage Forms*

For tablets and sachets: Twenty tablets or five sachets were weighed, powdered and mixed well. A portion equivalent to 20 mg of (I), 25 mg of (II), 40 mg of (III) or 50 mg of (IV) was weighed and quantitatively transferred into 100-mL volumetric flasks using methanol. The flasks were sonicated for 30 min and the volumes were completed to the mark with methanol. The solutions were filtered into dry flasks. Aliquots of these solutions were then treated as described above under the general procedure.

For drops: An aliquot of 0.1 mL of the drops was diluted to 25 mL with methanol. The above stated general procedure was applied to determine the drug concentration.

RESULTS AND DISCUSSION

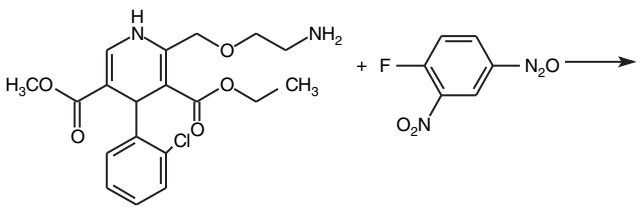
2,4-Dinitrofluorobenzene (DNFB) has been used in pharmaceutical analysis for the determination of specific functional groups such as primary and secondary amines, phenols, thiols and imidazoles[37]. In the present work DNFB reacts through a nucleophilic aromatic substitution reaction with thiols as (I) and (II) and amines as (III) and (IV) in aqueous alkaline medium to form yellow colored products as proposed in equations (a-c).



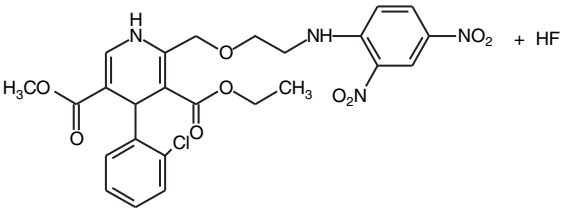
Thiols

2,4-Dinitrophenyl sulphide derivatives

(a)

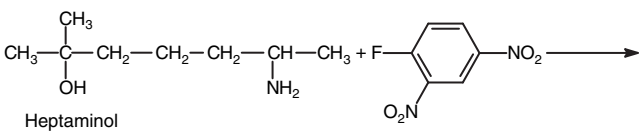


Amlodipine

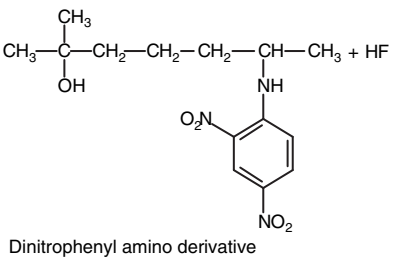


Dinitrophenyl amino derivative

(b)



Heptaminol



Dinitrophenyl amino derivative

(c)

The reported applications of this reaction to determine amine acid salts involved prior liberation of the base by alkalinization and subsequent organic solvent extraction of the basic drug. This step was omitted in the present study.

Heptaminol molecule contains a primary amino group of sufficient basicity to attack the electron deficient polynitro aromatic compound DNFB, while amlodipine features a primary aliphatic amino group and a dihydropyridine nitrogen which is neutral and is therefore a poor candidate for the reaction. The reaction stoichiometry between this latter drug and the reagent has been ascertained by applying the mole ratio method. The results obtained indicate a molar ratio of 1:1 thus confirming the participation of only the primary amino group in the reaction (Figure 1). The absorption spectra of the yellow colored products formed at the optimum conditions show characteristic λ_{max} as reported in Table 1. The experimental conditions were established by varying each parameter individually and noting its effect on the absorbance of the products.

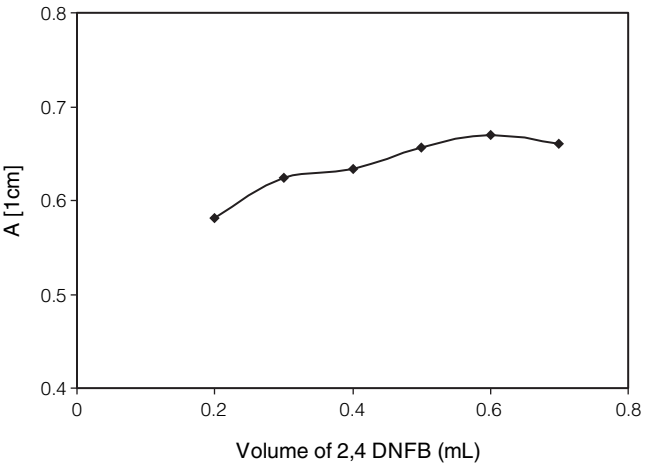


Figure 1. Molar ratio plot for amlodipine besylate and 2,4-dimitroflu orobenzene.

I. *Matrix Effects*

The reaction was investigated over the pH range of 7.4-9.4 using borate buffer. The products showed the highest absorptions in buffer of pH 7.8, 8, 7.8 and 8.5 for compounds (I), (II), (III) and (IV), respectively (Figures 2 and 3).

To remove excess reagent interference in the absorbance measurement of the reaction products, this excess was acid-hydrolyzed to colorless 2,4-dinitrophenol. The volume of acid (5 M hydrochloric acid) necessary to accomplish this task was determined (Table 1).

The optimum volumes of the buffer solutions were found to be 0.5 mL for the two thiols and 0.4 mL for the two amino compounds (Figures 4 and 5).

Effect of the reagent concentration on color development was also studied where it was found that 0.2 mL of 0.2% w/v reagent was sufficient to give the maximum color

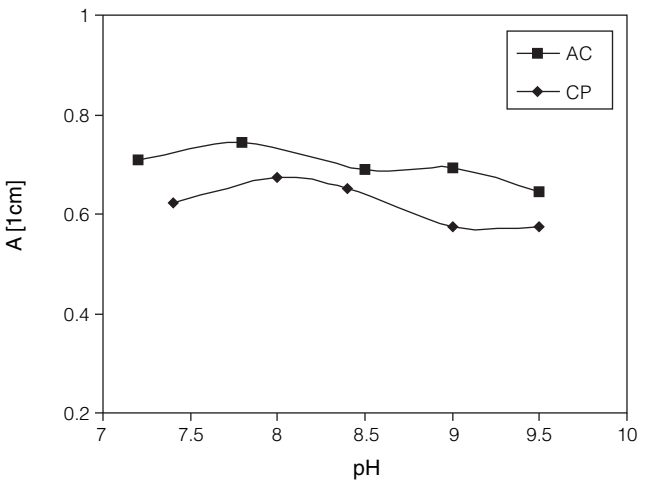


Figure 2. Effect of buffer pH on the substitution reaction of acetylcysteine (11.14 μg mL^{-1}) and captopril (14.01 μg mL^{-1}) with 2,4-dinitrofluorobenzene.

Table 1. Assay parameters for the determination of acetylcysteine (I), captopril (II), amlodipine besylate (III) and heptaminol hydrochloride (IV) by the proposed method

	Drug			
Item	I	II	III	IV
Standard conc. (mg mL^{-1})	0.2	0.24	0.4	0.5
Volume of standard solution (mL)	0.2-0.6	0.1-0.7	0.2-0.7	0.1-0.5
Borate buffer pH	7.8	8.0	7.8	8.5
Borate buffer volume (mL)	0.5	0.5	0.4	0.4
DNFB conc. (g %)	0.5	0.2	0.5	0.5
DNFB volume (mL)	0.3	0.2	1.0	0.8
Heating temperature (°C)	R.T.	40	60	40
Heating time (min)	30	20	25	40
5M HCl volume (mL)	0.1	0.1	0.2	0.1
λ_{max} (nm)	338	339	357	352

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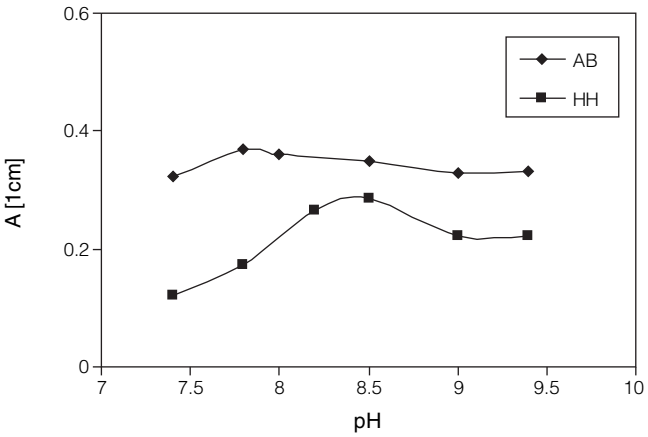

Figure 3. Effect of buffer pH on the substitution reaction of amlodipine besylate (10.09 μg mL^{-1}) and heptaminol hydrochloride (7.61 μg mL^{-1}) with 2,4-dinitrofluorobenzene.

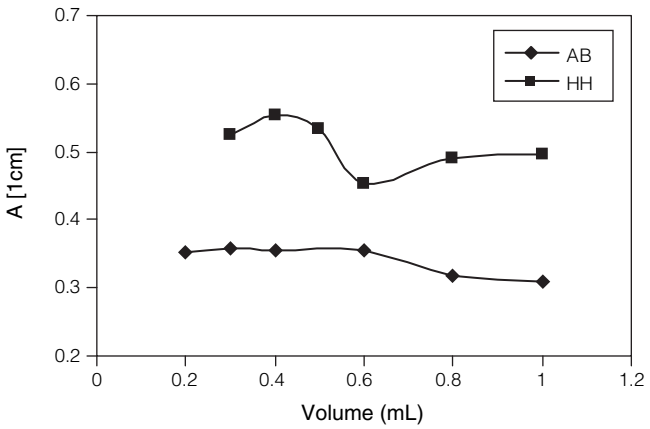

Figure 5. Effect of borate buffer volume on the substitution reaction of amlodipine besylate (9.64 μg mL^{-1}) and heptaminol hydrochloride (15.22 μg mL^{-1}) with 2,4-dinitrofluorobenzene.

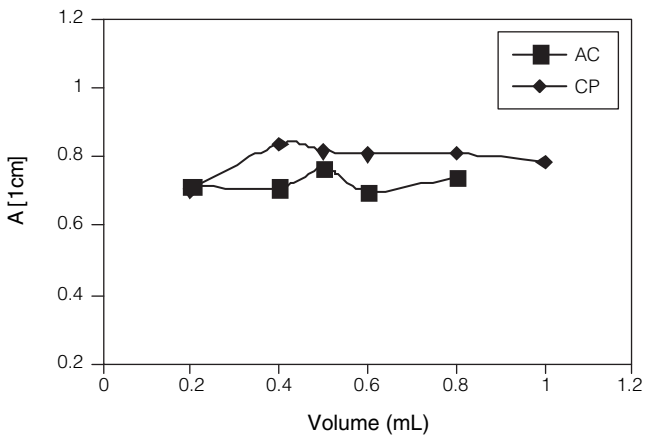

Figure 4. Effect of borate buffer volume on the substitution reaction of acetylcysteine (11.49 μg mL^{-1}) and captopril (16.81 μg mL^{-1}) with 2,4-dinitrofluorobenzene.

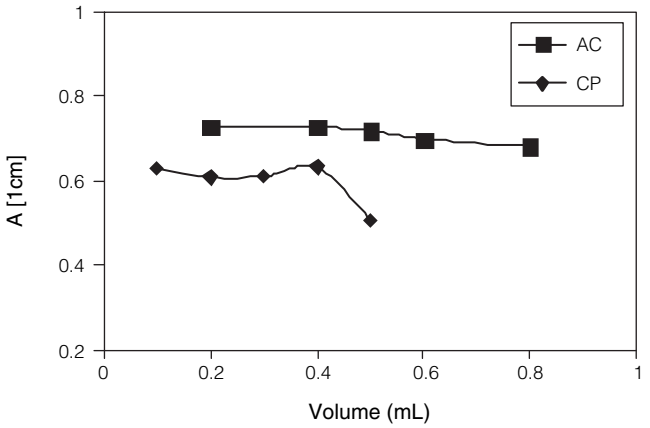

Figure 6. Effect of 2,4-dinitroflurobenzene (0.5% and 0.2% respectively) volume on its substitution reaction with acetylcysteine (10.9 μg mL^{-1}) and captopril (12.68 μg mL^{-1}).

intensity for compound (II), whereas 0.3, 1 and 0.4 mL of 0.5% w/v reagent gave the maximum sensitivity for drugs (I), (III) and (IV), respectively (Figures 6 and 7).

In order to obtain the highest and most stable absorbances, the effect of the reaction time and heating temperature was investigated (Figures 8-11). The optimal values are presented in Table 1. For compound (I), the reaction proceeds at room temperature and attains the maximum sensitivity after 30 min. Moreover, heating resulted in lack of reproducible results. For compound (II), heating at 40°C for 20 min led to the maximum color formation. Compound (III) required heating at 60°C for 25 min , while (IV) needed heating at 40°C for 40 min to give full color intensity. The reaction products were stable for at least 30 min.

II. *Validation of the Method*

The proposed method was validated according to the USP 27[38] criteria.

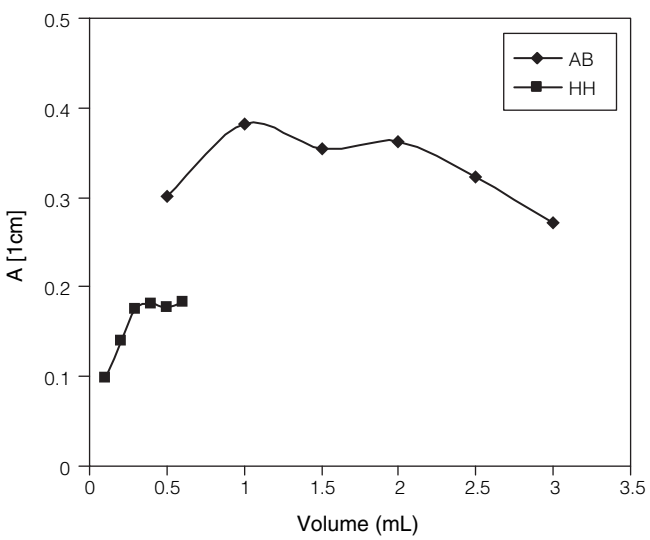

Figure 7. Effect of 2,4-dinitroflurobenzene (0.5%) volume on its substitution reaction with amlodipine besylate (10.48 μg mL^{-1}) and heptaminol hydrochloride (9.79 μg mL^{-1}).

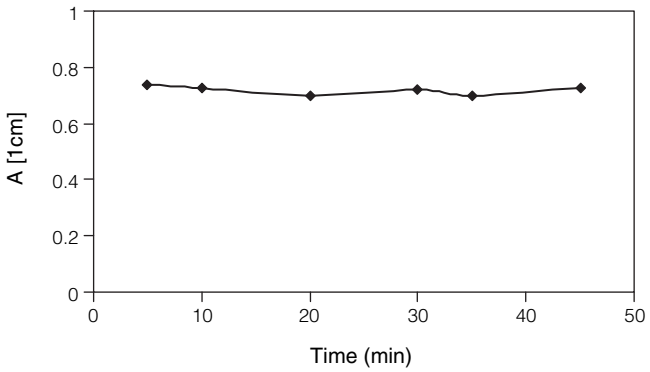

Figure 8. Effect of reaction time at room temperature on the substitution reaction of acetylcysteine (9.79 μg mL^{-1}) with 2,4-dinitro fluorobenzene.

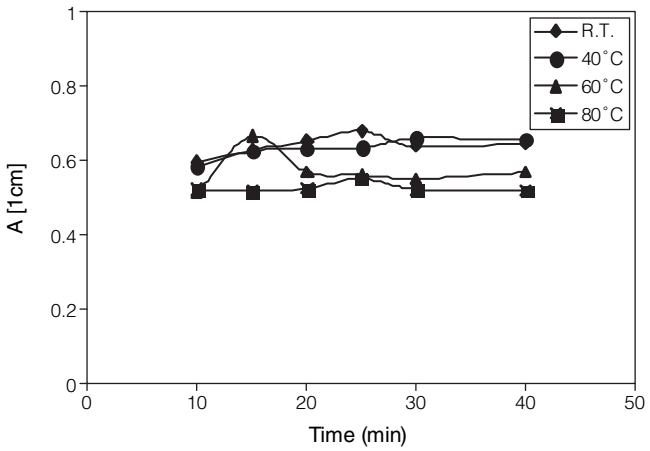

Figure 9. Effect of heating temperature and time on the substitution reaction of captopril (13.5 μg mL^{-1}) with 2,4-dinitrofluorobenzene.

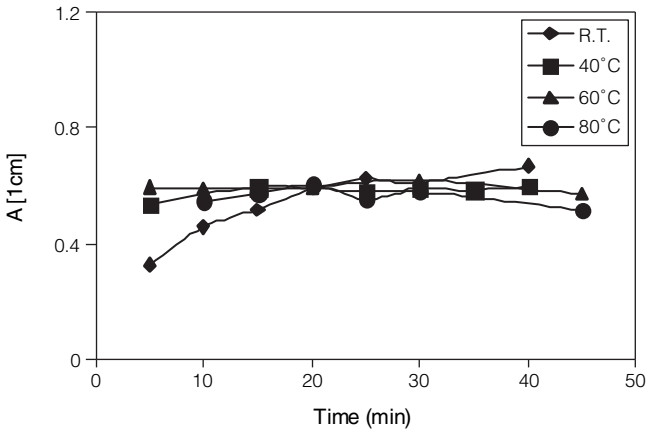

Figure 10. Effect of heating temperature and time on the substitution reaction of amlodipine besylate (17.69 μg mL^{-1}) with 2,4-dinitrofluor obenzene.

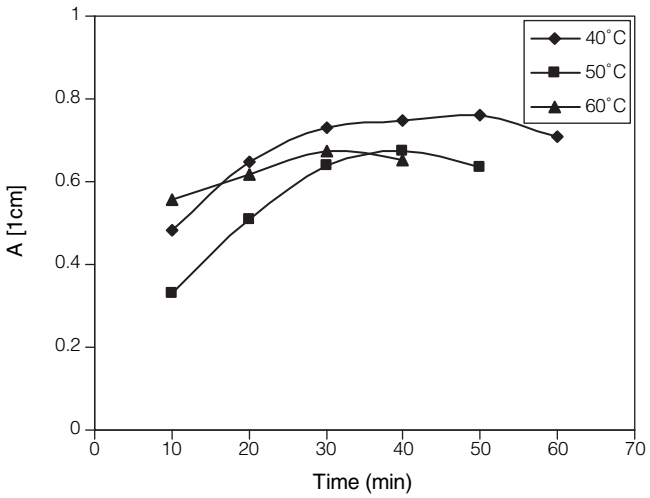

Figure 11. Effect of heating temperature and time on the substitution reaction of heptaminol hydrochloride(9.79 μg mL^{-1}) with 2,4-dinitrofl uorobenzene.

(I) *Linearity*

Linear correlations were found between the absorbance values and the concentration of the drugs over the ranges stated in Table 2. The good linearity of the calibration graphs is evidenced by low variances around the slopes and high correlation coefficients (Table 2).

(II) *Accuracy*

The accuracy was checked by calculating the recovery of the drugs spiked to the pharmaceutical preparations where good results were obtained (Table 2).

(III) *Precision*

In order to evaluate the precision of the proposed method, solutions containing three different concentrations of each drug were prepared and analyzed in three replicates. The mean relative standard deviations are presented in Table 2.

(IV) *Limit of Detection and Limit of Quantitation*

The detection and quantitation limits were calculated from the standard deviation of the absorbance measurements obtained from a series of blank solutions for each drug and are presented in Table 2.

III. *Analytical Applications*

The proposed method was successfully applied to determine the selected drugs in their pharmaceutical preparations. The results obtained were statistically compared to those of official or reported methods[32,39,40] by the student's t-test for accuracy and the variance ratio F-test for precision as recorded in Table 3. The experimental values of t and F did not exceed the theoretical values, indicating lack of significant difference between

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Table 2. Validation data for the determination of acetylcysteine (I), captopril (II), amlodipine besylate (III) and heptaminol hydrochloride (IV) by the proposed method

	Drug			
Item	I	II	III	IV
λ_{max} (nm)	338	339	357	352
Concentration range (μg mL^{-1})	4-12	2.4-16.8	8-28	5-25
Regression equation[a]				
Intercept (a)	-3.5×10^{-2}	1.8×10^{-3}	3.6×10^{-2}	1.7×10^{-2}
Variance of intercept (S_a^2)	7.5×10^{-5}	3.7×10^{-5}	2.5×10^{-5}	7.1×10^{-5}
Slope (b)	7.0×10^{-2}	4.8×10^{-2}	3.3×10^{-2}	3.5×10^{-2}
Variance around slope (S_b^2)	1.0×10^{-6}	3.0×10^{-7}	6.8×10^{-8}	2.0×10^{-7}
Correlation coefficient (r)	0.9996	0.9998	0.9999	0.9997
Variance ($S^2_{y.x}$)	4.1×10^{-5}	4.0×10^{-5}	1.9×10^{-5}	5.1×10^{-5}
Accuracy (mean ± SD)	100.70 ± 1.25	99.61 ± 0.92	100.73 ± 1.05	99.83 ± 1.07
Precision (RSD%)	1.48	1.20	2.02	1.81
Limit of detection (μg mL^{-1})	0.97	0.38	0.58	1.45
Limit of quantitation (μg mL^{-1})	3.24	1.27	1.93	4.85

[a]A = a + bC, where C is the concentration in μg mL^{-1} and A is absorbance unit.

Table 3. Assay results of drugs (I), (II), (III) and (IV) in their pharmaceutical preparations using the proposed method

Preparation	Supplier	Labeled amount	Recovery % ± SD[a]	Reference method[32,39,40]
Acetylcistein instant effervescent powder	South Egypt Drug Industries Co.	200 mg/sachet	101.94 ± 1.11 t = 1.59 F = 3.00	102.85 ± 0.64
CapotenTM tablets	Bristol Myers Squibb, Egypt	25 or 50 mg captopril/tab	100.50 ± 1.45 t = 2.15 F = 6.24	101.99 ± 0.54
Capozide tablets	Bristol Myers Squibb, Egypt	25 mg captopril and 12.5 mg hydrochlorothiazide/tab	100.90 ± 1.62	
Norvasc tablets	Pfizer, Egypt	The equivalent of 5 mg amlodipine/tab	99.28 ± 0.46 t = 2.02 F = 2.01	100.0 ± 0.65
Lotrel tablets*	Novartis, USA	The equivalent of 5 mg amlodipine and 10 or 20 mg benazepril/tab	100.87 ± 0.76	
Corasor tablets	Amoun Pharmaceutical Co., Egypt	150 mg heptaminol hydrochloride/tab	101.15 ± 1.33 t = 0.51 F = 1.92	101.67 ± 1.84
Respirin drops	Pharco Pharmaceuticals, Egypt	150 mg heptaminol hydrochloride/mL	99.37 ± 0.83 t = 2.06 F = 1.39	98.36 ± 0.71

[a]Each value is the mean of five measurements.
[b]Theoretical values for t- and F- at p = 0.05 are 2.31 and 6.39, respectively.
[32]Hantzch condensation reaction method for heptaminol.
[39]The BP- HPLC- method of captopril for both acetylcysteine and captopril.
[40]Reversed-phase HPLC method for amlodipine.
*Laboratory prepared tablets as documented in the Physician Desk Reference (PDR).

the compared methods. Based on the data in Table 3, it was found that amino compounds demonstrated better results in the proposed method compared to those of the reference methods concerning the values of the SD. However, the thiols demonstrated relatively less satisfactory results. This may be attributed to the greater nucleophilicity of the amino compounds than the thiols; therefore, reaction in the former case goes more smoothly resulting in better reproducibility and hence better values of standard deviations. Furthermore, applicability of the developed method to multi-component pharmaceutical formulations has been verified by analyzing captopril in the presence of hydrochlorothiazide and amlodipine in combination with benazepril where no interference was made by these common co-formulated drugs as evidenced by the good recoveries obtained. The results are shown in Table 3.

CONCLUSIONS

The proposed method can be recommended for routine quantitative determination of the studied drugs in quality control laboratories where modern equipments are unavailable. The absence of interference from added excipients, additives and some co-formulated drugs is a noted advantage. Another advantage was simplicity of the method as evidenced by direct application of the reaction to the amine salts like amlodipine besylate and heptaminol hydrochloride without the need of prior conversion to the base and subsequent extraction.

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