SHORT REPORT
doi: 10.2306/scienceasia1513-1874.2008.34.107

ScienceAsia 34 (2008): **107-113**

Kinetic Spectrophotometric Determination of Acetylcysteine and Carbocisteine in Bulk Powder and in Drug Formulations

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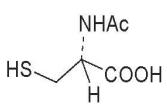
Received 2 Feb 2007
Accepted 3 Sep 2007

ABSTRACT: A simple, sensitive and validated kinetic spectrophotometric method for the determination of certain mucolytic drugs namely acetylcysteine (Ac) and carbocisteine (Cc) is described. This method is based on the kinetic investigation of the reaction between the two cited drugs and 4-chloro-7-nitrobenzo-2-oxa 1, 3 -diazole (NBD-Cl) in an alkaline medium. Spectrophotometric measurements were achieved by recording the absorbance at 424 nm at ambient temperature (25^0C ±5) for a fixed time of 30 minutes for Ac, and at 468 nm for a fixed time of 15 minutes at 70 ^{0}C for Cc. All variables affecting the development of the reaction were investigated and optimized. The method was found to be linear over the range of 2-22 μg ml^{-1} for Ac, and 5-35 μg ml^{-1} for Cc with mean percentage recoveries of 99.59 ± 0 .41 and 99.50 ± 0.65, respectively. Molar absorptivity and sensitivity index were determined and found to be ($1.094x10^3$and $1.49x10^{-2}$, respectively, for Ac, and $2.547x10^3$ and $3.05x10^{-2}$, respectively for Cc. The validity of the method was assessed according to USP guidelines. The proposed method was successfully applied for the determination of the two cited drugs in bulk powder, in pharmaceutical formulations as well as in the presence of their related substances. The results obtained were found to agree statistically with those obtained by official and reported methods. In this study the determination of the two drugs by the fixed time method proved to be more applicable than the fixed absorbance and rate constant methods.

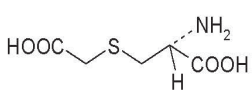
KEYWORDS: Kinetic determination, Acetylcysteine (Ac), Carbocisteine (Cc), 4-chloro-7-nitrobenzo-2-oxa 1,3 -diazole (NBD-Cl).

INTRODUCTION

Acetylcysteine (Ac, N-acetyl-3-mercaptoalanin) and carbocisteine (Cc, (R)-2-amino-3-{(carboxymethyl) thio} propionic acid) are two biologically active substances used as mucolytic drugs[1] , Ac is also used as antidote for paracetamol poisoning. [2] They have the following structures.



Acetylcysteine (Ac)



Carbocisteine (Cc)

British Pharmacopoeia describes an iodimetric method for the determination of Ac and a non aqueous titrimetric method for the determination of Cc[3] which lack accuracy and sensitivity. Different analytical methods have been published for their determination. The most commonly used methods are spectrophotometric[4-6], spectrofluorimetric[7-9], chromatographic[10-12] and electrochemical methods [13-15].

Kinetic-based analytical methods for drug analysis are not widely applied although they offer the advantage of eliminating additive interference, which probably affects other methods such as the titrimetric and direct spectrophotometric ones.

The kinetic investigation, based on a kinetic spectrophotometric method, of the derivatization reaction between each of Ac and Cc and 4- chloro 7-nitrobenzo-2-oxa 1,3 - diazole (NDB-Cl) in an alkaline medium at 424 nm for Ac and 468 nm for Cc is proposed, and applied for the determination of the two drugs. The molar ratio of the reaction was determined and the mechanism was suggested. The applicability of the developed method was evaluated through the determination of both drugs in bulk powder, and in laboratory prepared mixtures containing different percentages of their related substances (L-cystine and L-cysteine of Ac, and L- arginine for Cc).

MATERIALS AND METHODS

Reagents, Chemicals and Standard solutions

All chemicals and solvents were of analytical grade.

Reference samples

Ac and Cc were kindly supplied by (Sideco. Co. Egypt) and assayed for purity according to the official titrimetric methods[3] to contain 99.22 ± 0.28% and 99.26 ± 0.52 % for both drugs, respectively. L-cystine (Otsoka .Co. Egypt.) certified to be 99 .50%. L-cysteine (Otsoka Co. Egypt.) certified to be 99.50%. L-arginine (Otsoka Co. Egypt.) certified to be 99.50%.

Market samples

Parvolex ampoules (2 gm Ac/10 ml), B.N.13140, were purchased from Celletch Pharmaceuticals Limited, U.K. Mucosol capsules (375 mg of Cc), B.N.11966 MUP. Co .Egypt.

Chemicals

NBD-Cl (Sigma) 0.1% (w/v) solution in acetone for Ac, and 0.4% (w/v) solution in methanol for Cc. Both solutions should be freshly prepared. Borax (El-Nasr pharmaceutical Chemicals, Co. Egypt.), $5x10^{-3}$ M. aqueous solution. Borate buffer pH9 was prepared according B.P.2007 [3]

Stock solutions

(0.20 mg ml^{-1}) of Ac in water, and (0.50 mg ml^{-1}) of Cc in 0.001M NaOH. The solutions were used within three days if stored at $4C^0$ protected from light .

Apparatus

Spectrophotometric measurements were carried out using a double beam UV-Visible spectrophotometer (SHIMADZU, Japan) Model UV. 1601 PC, connected to an IBM compatible computer and HP 800 ink-jet printer. The bundle software was a UVPC personal spectroscopy software version 3.7. The spectra band width was 2 nm and wave length scanning speed was 2800 nm min^{-1}.

Recommended Procedures

For Ac

Aliquots equivalent to 20-220 µg Ac were transferred into a series of 10-ml volumetric flasks. To each flask 1ml of 5×10^{-3} M borax solution and 1.5 ml of 0.1 % NBD-Cl solution (in acetone) were added. The volume was completed to the mark with distilled water. The absorbance of the resulting solutions was measured at 424 nm at 5 minutes intervals for 30 minutes at ambient temperature ($25^0C \pm 5$) against a blank solution prepared simultaneously.

For Cc

Aliquots equivalent to 50-350 µg were transferred into a series of 20-ml test tubes. To each tube 1.5 ml of borate buffer, pH 9 and 0.5 ml of 0.4% NBD-Cl (in methanol) were added, and allowed to stand in a thermostatically controlled water bath at 70 ^0C for 15 minutes. The mixture was cooled, transferred quantitatively to a 10-ml volumetric flask and completed to the mark with distilled water. The absorbance of the resulting solutions was measured at 464 nm at 3-minutes intervals for 15 minutes against a blank solution prepared in the same manner.

The absorbance values were plotted against drug concentrations after 30 and 15 minutes, and the regression equations were computed for both drugs.

Analysis of Pharmaceutical Formulations

Ampoules

The contents of three ampoules were mixed well in 50 ml dried beaker. Aliquots equivalent to 20 mg of Ac were transferred into 100-ml measuring flasks and diluted with distilled water to the volume. The determination of Ac proceeded as directed under general procedures.

Capsules, the contents of 10 capsules were mixed thoroughly, an accurate weight equivalent to 20 mg Cc was transferred into a 100-ml conical flask, and 0.5 ml of 0.1 M NaOH and 50 ml water were added. This mixture was sonicated for 25 minutes, filtered, transferred quantitatively to a 100-ml measuring flask and completed to the volume. The determination of Cc proceeded as directed under general procedures.

Results

Ac and Cc were determined using a spectrophotometric method based on their derivatization reaction with 4-chloro 7- nitrobenzo-2-oxa 1,3 diazole (NBD-Cl) in alkaline medium to form yellow colored reaction products with maximum absorbance at 424 ± 2 nm for acetylcysteine and at 468 ± 2 nm for carbocisteine as shown in Fig. 1.

At room temperature ($25^0C \pm 5$) the reaction between Ac and NBD-Cl increased substantially with time, while for Cc the reaction increased at ($70^0C \pm 5$) as revealed by the intensification of the developed color and subsequent increase in the slope of the calibration graphs shown in Table 1.

The rate of the reactions was found to be dependent on the concentration of each of Ac and Cc. For Ac the rates were followed at room temperature for the

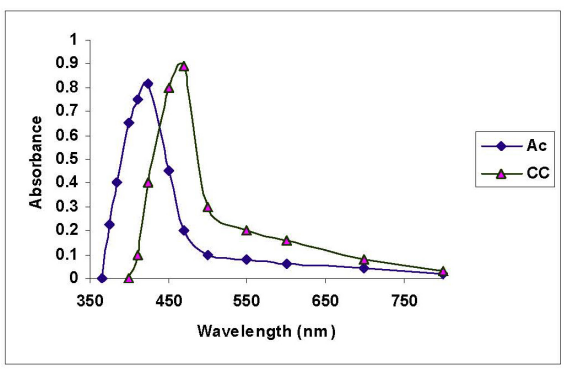


Fig 1. Absorption spectra of reaction products between Ac (12 ug ml^{-1}) and Cc (25 ug ml^{-1}) with NBD-Cl.

Table 1. Calibration equations at different fixed times for Ac in the range of 2-22 μg ml^{-1} and Cc in the range of 5-35 μg ml^{-1}.

Correlation coefficient(r)	Calibration equation	Time (min)
	Ac	
0.9859	A=0.0490c-0.01613	5
0.9976	A=0.0619c-0.03990	10
0.9988	A=0.0687c-0.03390	15
0.9986	A=0.0731c-0.04930	20
0.9988	A=0.0752c-0.49400	25
0.9989	A=0.0763c-0.49000	30
	Cc	
0. 9858	0.00998c+0.0201	3
0.9977	0.01736c+0.0408	6
0.9988	0.02518c+0.0285	9
0.9994	0.02974c+0.0205	12
0.9996	0.03160c+0.0172	15

specified time at the specified concentration range (2-22 μg ml^{-1}) keeping NBD-Cl and borax constant at high concentration, and at 70^{0}C in the range (5-35μg ml^{-1}), keeping NBD-Cl, ml borate buffer pH 9 constant at high concentration for Cc as shown in Figs 2,3.

The rate of the reaction was estimated by the variable time method measured as ΔA/Δt, where A is the absorbance and t is the time in seconds as shown in Table 2.

The fixed time method was applied for determination of the two cited drugs in bulk powder as shown in Table 3 and in pharmaceutical formulations, the obtained results are shown in Table 4.

Discussion

NBD-Cl is a highly sensitive and selective chromogenic and fluorogenic reagent which is used for derivatization of aliphatic thiols, and primary, secondary and tertiary amines.

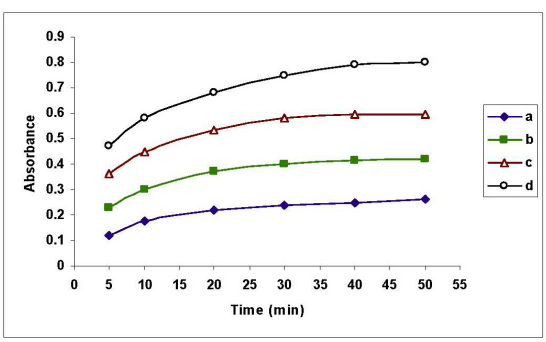


Fig 2. Graph of absorbance versus time for Ac-NBD-Cl reaction showing the dependence of the reaction on Ac concentration : a) 2.449x10^{-5} M, b) 3.674x10^{-5} M, c) 4.898x10^{-5} M and d) 7.348x10^{-5} M.

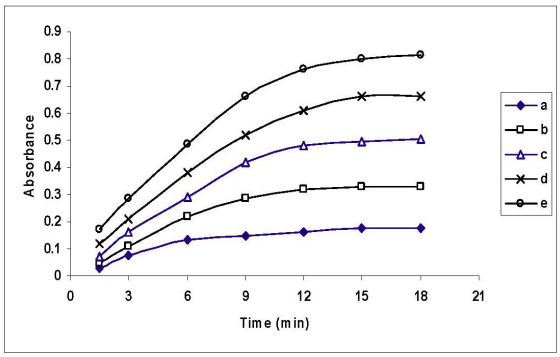


Fig 3. Graph of absorbance versus time for Cc-NBD-Cl reaction showing the dependence of the reaction on Cc concentration: a) 36.313x10^{-5} M, b) 12.626x10^{-5} M, c) 18.939x10^{-5} M, d) 25.252x10^{-5} M and e) 31.565x10^{-5} M.

Table 2. Logarithms of the rates for different concentrations of Ac and Cc.

Log concentration (mol L^{-1})	Log rate, log ΔA/Δt
Ac	
-4.61	-4.63
-4.34	-4.44
-4.31	-4.30
-4.13	-4.10
-4.01	-3.97
-3.96	-3.90
Cc	
-4.20	-3.96
-3.90	-3.63
-3.72	-3.41
-3.60	-3.27
-3.50	-3.18

Table 3. Analytical parameters for the determination of Ac and Cc in bulk powder by applying the fixed time method.

Parameter	Cc	Ac
λ nm	468	424
Linearity range(μgml^{-1})	5-35	2-22
Molar absorbability	2.547x10^3	1.094x10^3
Regression equation		
Intercept (a)	0.0081	-0.0096
S.E. of intercept	0.0048	0.0078
Slope (b)	0.0322	0.0067
S.E. of slope	0.0002	0.0006
Correlation coefficient (r)	0.9996	0.9998
Standard error of estimation (S.E)	0.0072	0.0123
Accuracy (mean ± RSD%)	99.85±0.251	99.77± 0.168
Precision (RSD%)		
a) interday precision	100.15± 0.266	a) 99.95± 0.285
b) intraday precision	99.88 ± 0.508	b) 99.92± 0.495

Table 4. Statistical comparison of the results obtained for the determination of Ac in Prvolex ampoules and Cc in. Mucosol capsules using the fixed time method with those obtained by official[3] and reported methods[21].

Pharmaceutical Preparations	Reference methods	%Recovery	µg found	µg taken
Parvolex ampoule 2gm Ac/10ml				
		98.32	1.97	2
		99.89	3.99	4
		100.04	6.00	6
		98.01	11.76	12
		101.74	14.24	14
Mean*±RSD	100.18±1.6[3]	99±1.50		
Student's t value		0.607(1.895)[a]		
Variance ratio F test		1.185(6.4)[a]		
Mucosol capsule375 mg Cc				
		97.45	4.88	5
		99.66	9.97	10
		99.98	14.99	15
		99.52	19.90	20
		99.49	24.87	25
Mean*±SD	99.22±1.01[21]	98.36±1.19		
Student's t value		1.227(1.895)		
Variance ratio F test		1.396(6.4)		

* Mean of 5 different experiments
[a] Theoretical values

Being saturated aliphatic compounds, Ac and Cc don't possess UV absorption spectra. A spectrophotometric method was developed for their determination based on their derivatization reaction with NBD-Cl in an alkaline medium to form yellow products with a maximum absorbance at 424 ± 2 nm for Ac, and at 468 ±2 nm for Cc (Fig.1).

The extent of the formation of these species depends on the concentration of the reactants, alkalinity, temperature and time; therefore the effects of these variables were carefully studied.

For Ac

Effect of pH

Different aqueous bases such as borax, sodium bicarbonate and dibasic sodium phosphate were tried. Also different volumes of borate buffer, pH 8.4 (using $5x10^{-3}$ M borax) were tried. The best results were obtained upon using 1ml of borax solution ($5x10^{-3}$ M).

Effect of reagent concentration

Different concentrations of NBD-Cl ranging from 0.05- 0.15 % w/v in methanol and in acetone were tried. Also different volumes of 0.1% w/v NBD-Cl solution in acetone ranging from 0.25- 2 ml were used. It was found that 1.5 ml of 0.1% w/v NBD-Cl solution in acetone was sufficient for the production of maximum and reproducible color intensity .

Effect of temperature and time

At room temperature (25^0C ± 5), the reaction increased substantially with time, as revealed by the intensification of the developed color and subsequent increase in the slope of the calibration graphs (Table 1), indicating high analytical sensitivity. Therefore, the reaction time of 30 minutes at room temperature was selected as the optimum reaction time .

For Cc

Effect of pH

Different buffers such as borate and phosphate buffers were tried. Also borate buffer of different pH range (7.5-11) were used. The best results were obtained upon using 1.5 ml of borate buffer, pH 9 .

For both drugs in an acidic medium, no reaction took place, while in a strong alkaline medium the absorbance of the blank solutions increased.

Effect of reagent concentration

Different concentrations of NBD-Cl ranging from 0.1-0.5% w/v in methanol and in acetone were tried, and different volumes of 0.4% w/v solution in methanol ranging from 0.1- 0.7 ml were also tried. It was found that 0.5 ml was suitable for producing maximum color intensity .

Effect of temperature and time

At room temperature (25^0C ± 5), the reaction of Cc with NBD-Cl was very slow. To obtain complete color development, the temperature was raised to 70^0C ± 5 on a thermostatically controlled water bath for a fixed time of 15 minutes. Below this temperature the reaction is not completed, while above this temperature the color intensity of the product is diminished. The solution was kept for different time intervals in a thermostatically controlled water bath at 70^0C ± 5 from 3-20 min. The maximum stable absorbance was obtained after heating at 70 ^{0}C for 15 min as revealed by subsequent increase

in the slope of the calibration graphs.

Effect of diluting solvents

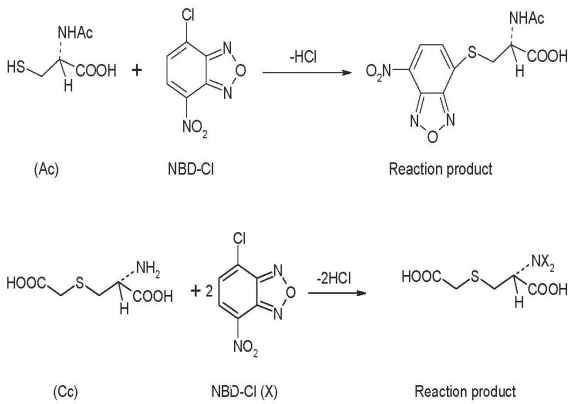
For both drugs, different diluting solvents such as distilled water, methanol, ethanol, isopropyl alcohol and acetone were investigated. Water and methanol were found to be the solvents that gave the best sensitivity. There was no great difference in absorbance upon using water and methanol. Therefore, for economic and environmental protection purposes, water was used as the best diluting solvent.

Effect of time on the stability of the reaction products

The absorbance of the colored adduct remained stable for at least 2 hours for both drugs.

The stoichiometric ratio between each of Ac and Cc to NBD-Cl was determined using the limiting logarithmic method[16] . The ratios were found to be (1:1) for Ac and (2:1) for Cc.

The following reaction mechanisms are proposed to take place between NBD-Cl and the two cited drugs.



Scheme 1: The suggested mechanisms of the reaction of Ac and Cc with NBD-Cl.

Kinetic Study of the Reaction

The rate of the reactions was found to be dependent on the concentration of each of Ac and Cc. For Ac, the rates were followed at room temperature for the specified time at the specified concentration range (2-22 $\mu g\ ml^{-1}$) keeping NBD-Cl and borax constant at high concentration, and at 70^0C in the range (5-35 $\mu g\ ml^{-1}$), while keeping NBD-Cl, ml borate buffer pH 9 constant at high concentration for Cc, as described under the general procedure.

From the graphs shown in Figs.2, 3, the rate increased as Ac and Cc concentrations increased indicating that the reactions rates obey the equation:

$$\text{Rate} = K^- [\text{Ac}]^n \quad (1)$$

$$\text{Rate} = K^- [\text{Cc}]^n \quad (2)$$

Where K^- is the pseudo first-order rate constant of the reaction and n is the order of the reaction. The rate of the reaction may be estimated by the variable time method measured as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds[17] . Taking logarithms of rates and concentrations, as shown in Table 2, equations 1 and 2 are transformed into:

$$\text{Log (Rate)} = \text{Log}(\Delta A/\Delta t) = \text{Log } K^- + n \text{ Log [Ac]}. \quad (3)$$

$$\text{Log (Rate)} = \text{Log}(\Delta A/\Delta t) = \text{Log } K^- + n \text{ Log [Cc]}. \quad (4)$$

Regression of Log (Rate) versus Log Ac gave the following regression equation:

Log (Rate) = 1.1117 Log c +0 .4939 (r = 0.9998)

While regression of Log (Rate) versus Log Cc gave the following regression equation:

Log (Rate) = 0.8809Log c -0.7092 (r = 0.9995)

Hence $K^- = 0.1977\ s^{-1}$ for Cc and $3.1103\ s^{-1}$ for Ac. The reaction can be approximated to first order (n = 1) with respect to Ac and Cc concentrations.

Evaluation of the Kinetic Methods

The quantitative determination of Ac and Cc under the optimized experimental conditions outlined above would result in a pseudo-first order reaction with respect to their concentrations. The concentration of NBD-Cl was at least 30 times of the initial concentration of Ac and 26.7 times the initial concentration of Cc. Borax concentration was at least 17 times the initial concentration of Ac. However the rates will be directly proportional to Ac and Cc concentrations in a pseudo-first order rate equations as follows:

$$\text{Rate} = K\,[\text{Ac}] \quad (5)$$

$$\text{Rate} = K\,[\text{Cc}] \quad (6)$$

Where K is the pseudo-first order rate constant. Equations (5) and (6) were the basis for several experiments which were carried out to obtain Ac and Cc concentrations. The rate constant, fixed-concentration and fixed-time methods [18,19] were tried and the most suitable analytical method was selected, taking into account the applicability, sensitivity, the slope of the calibration curves, correlation coefficient (r) and the intercept.

Rate Constant Method

Graphs of log (absorbance) versus time (sec) over the concentration range of 2.45×10^{-5} to 12.25×10^{-5} mol L^{-1} for Ac, and 6.313×10^{-5} to 31.565×10^{-5} mol L^{-1} for Cc, were plotted .The pseudo-first order rate constants corresponding to different Ac and Cc concentrations were calculated from the slopes multiplied by -2.303.

Regression of concentration (c) versus K gave the following equations:

$K = 0.2982\ c + 1.86 \times 10^{-4}$ (r = 0.9724) for Ac

$K = -9.686 \times 10^{-4} c - 1.26 \times 10^{-3}$ (r = 0.9017) for Cc

The values of r are indicated of poor linearity probably because of inconsistency of K.

Fixed Absorbance Method

Reaction rates were recorded for different Ac and Cc concentrations in the range of $2.45\ x10^{-5}$-12.25

$x10^{-5}$ mole L^{-1} for Ac, and 6.131 $x10^{-5}$ - 31.565 $x10^{-5}$ mol L^{-1} for Cc. A pre-selected values of the absorbance for both drugs (0.84 and 0.40, respectively) were fixed, and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of Ac and Cc were plotted, and the following equations for the calibration graphs were obtained by linear regression.

$1/t = 0.0146c+2.530x10^{-5}$ (r = 0.9966) for Ac.

$1/t = 0.549c+1.296x10^{-4}$ (r = 0.9953) for Cc.

The ranges of concentration giving the most acceptable calibration graph with the above equations were limited to 8 - 12 μg ml^{-1} for Ac, and 15 - 25 μg ml^{-1} for Cc, which could be a disadvantage .

Fixed Time Method

Reaction rates were determined for different concentrations of Ac and Cc. At a pre-selected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus the initial concentrations of Ac and Cc were obtained at fixed times of 5, 10, 15, 20, 25, 30 minutes for Ac, and 3, 6, 9, 12, 15 minutes for Cc, with the calibration equations shown in Table 1. It is clear that the slopes increased with time and the mot suitable values of the correlation coefficient (r) and the intercepts were obtained for fixed times of 30 and 15 minutes for Ac and Cc, respectively, which were therefore chosen as the most suitable time intervals for measurements.

Under the optimum experimental conditions, the calibration curves were plotted representing the relationship between the absorbance at 424 nm for Ac and at 468 nm for Cc and their corresponding concentration. Linear correlation coefficients were obtained. The concentration range was found to be 2-22 μg ml^{-1} and 5-35 μg ml^{-1} for Ac and Cc, respectively . Data analysis gave the following regression equations

$A = 0.0763c-0.049$ (r= 0.9989) for Ac

$A = 0.0316c+0.0172$ (r= 0.9996) for Cc

Analytical Application

The fixed time method was applied for the determination of both drugs in bulk powder (Table 3) and in pharmaceutical preparations. The obtained results are shown in Table 4.

Furthermore, it was also applied for the determination of the two cited drugs in the presence of their related substances, L-cysteine up to 5 % and L-cystine up to 90 % for Ac, and L-arginine up to 10 % for Cc. The concentration of Ac and Cc were calculated using the corresponding calibration equations shown in Table 1 at fixed time of 30 and 15 min for both drugs respectively. The results obtained for the analysis of both drugs in bulk powder and drug formulations were compared with those obtained with the official titrimetric[3] and reported[20] methods. The student t-test and F-test values at 95 % confidence limit did not exceed the theoretical values of 1.865 and 6.4 for t-test and F-test, respectively, indicating no significant difference between the performance of those methods regarding accuracy and prescion.

Method Validation

Linearity range

Under the experimental conditions, Beer's plot for the two cited drugs using the suggested method showed linear relationships with regression equations shown in Table 3.

Accuracy

The accuracy of the proposed method was determined by investigating the percentage recovery at five levels, each in triplicate, ranging from 4-20 and 10-30 μg ml^{-1} for PAc and Cc , respectively. The percentage relative standard deviation (RSD%) revealed high accuracy.

Precision

The intraday precision was evaluated by assaying freshly prepared solutions in triplicate at the concentration range of 8-18 and 15-35 ¼g ml^{-1} for Ac and Cc, respectively. The percentage relative standard deviations (RSD%) were found to be 99.95± 0.285 for Ac and 100.15± 0.266 for Cc. While the interday precision was calculated by assaying freshly prepared solutions in triplicate for three days. The relative standard deviations (RSD%) were found to be 0.495 for Ac and 0.508 for Cc.

Specificity

For the specificity determination, synthetic mixtures of different percentages of each drug and its related substance were prepared. It was found that Ac can be determined in the presence of its related substance, L-cysteine up to 5% and L-Cystine up to 90 %, while Cc can be determined in the presence of L-arginine up to 10 % .

Robustness

Two sets of experiments were carried out using two different calibrated spectrophotometers SHIMADZU 1601 UV/VIS and UNICAM UV 300 with 1 cm matched glass cells. Also two sets were carried out on SHIMADZU 1601 by two different analysts. No significant difference was obtained between the results in this study.

The validity of the suggested method was assessed by applying the standard addition technique by adding Ac and Cc to the previously analyzed pharmaceutical preparations. Comparison of the results obtained by the proposed method with those obtained by titremetric official method[3] for ampoules and reported HPLC method for capsules [20] , showed that the recommended procedures are more economical with regards to solvent and reagent consumption without any loss of accuracy or precision .

Conclusion

The kinetic-based method was sensitive, selective and accurate compared with the official titrimetric methods. Furthermore, it did not need the elaborate treatment and expensive column and solvents required in reported HPLC ones. The data given above revealed that the proposed method could determine Ac and Cc in the presence of their related substances with good prescion and accuracy. So with this method, one can do the analysis at low cost without losing accuracy. It can be used as an alternative method to the official and reported ones for routine determination of the two cited drugs in bulk powder and in pharmaceutical formulations.

References

1. Brunton LL, Lazo JS, Parker KL(2006) Goodman and Gilman' s "The Pharmacological Basis of Therapeutics" 11th ed., pp 694. McGraw-Hill, NewYork.
2. Sweetman SC (2005) Martindale "The Complete Drug Reference", 34th ed., pp1112, 1113, 1116. The Pharmaceutical Press, London.
3. British Pharmacopoeia, (2007) Vol. I, pp 50, 51, 376, 377, Her Majestys' Stationery Office, London.
4. Eid MA (1998) Spectrophotometric determination of cysteine and N- acetylcysteine in pharmaceutical preparation. *Mikrochim Acta*. **29**, 91-5.
5. Nedeljkovic JM, Vasic VM, Vukovic VV and Jovanovich TS (1995) Kinetic of the reaction of S- carboxymethyl- L-cysteine with palladium (II) chloride. *Pharm Biomed Anal.* **13**, 471-475.
6. Taha E A, Hassan N Y, AbdelAal F and AbdelFattah L S (2005) Spectrophotometric determination of certain mucolytic drugs in prescence of their related substances through oxidation with cerium and vanadium. *Bull Fac Pharm Cairo Univ* **43** , 22-26 .
7. Sano A, Takezawa M and Takitani S (1987) Fluorimetric determination of cyanide with naphthalene-2,3-dicarboxyaldehyde and taurine. *Talanta* **34**, 743-744
8. Gala B, Gomez-Hens A and Perez-Bendino D (1995) Use of a stopped flow/ T formate spectrofluorimeter for simultaneous kinetic analyses . *Anal Chim Acta* **310**(3), 453-459.
9. Taha EA, Hassan N, Y, AbdelAal F and AbdelFattah L S (2007) Fluorimetric determination of some sulfur containing compounds through complex formation with terbium and uranium . *J fluoresc* **17**(3) 293-300
10. Indrayanto G, Widjaja S, Aditama L and Darmawan L (1998) Densitometric determination of S- carboxymethyl- L- cysteine in syrup and valdation of the method. *Planar Chrom Mod TLC.* **11**, 263-266.
11. Toussaint B, Pitti C, Streel B, Ceccato A, Hubert P and Crommen J (2000) Quantitative analysis of N-acetylcysteine and its pharmacopeial impureties in pharmaceutical formulations by liquid chromatography- ultra- violet detection- mass spectrometry. *Chromatogr* **896**, 191-199.
12. Vander HydenY, Mangelings D, Van Brempt J and Spapen H (2004). Development and validation of an HPLC method with postcolumn derivatization for assay of N-acetylcysteine in plasma. *Acta Chromatogr* **14**, 149-164.
13. Kolar M and Dobcnik D (2003) Chemically prepared silver electrode for determination of N-acetylcysteine by flow injection potentiometry. *Pharmazie* **58**, 25-28.
14. Radic N and Komljenovic J (1998) Determination of N-acetylcysteine by flow injection potentiometry. *Fresenius J Anal Chem* **360**(6), 675-678.
15. Yan J L, Sun RD and Sun W D (2003) Electrochemical behaviour of N-acetylcysteine at mercury film electrode. *Fenxi-Huaxue* **31**, 448-450.
16. Rose, J. (1964) "Advanced Physicochemical Experiments" Pitam, London, 67.
17. Weisberger A, Friess S, Lewis E (1953) Techniques of organic chemistry, part 1. vol 3. Interscience New York.
18. Yatsimirskii K (1966) Kinetic Methods of Analysis. Pergamon, Oxford .
19. Laitinen H, Harris W (1975) Chemical Analysis, 2nd ed. McGraw-Hill, New York .
20. Manufacturer Method supplied by MUP. CO. Egypt, by personal communication (2005).