https://doi.org/10.15255/KUI.2024.046
KUI-15/2025
Original scientific paper
Received October 23, 2024
Accepted December 5, 2024

# Development and Validation of a Novel Kinetic Spectrophotometric Method for Determining *N*-acetyl-L-cysteine

*M. Biočić Šormaz*,[a*] *T. Kraljević*,[b] and *L. Kukoč-Modun* [a]

[a] Department of Analytical and Environmental Chemistry, Faculty of Chemistry and Technology, University of Split, Rudjera Boškovića 35, 21000 Split, Croatia
[b] Department of Chemistry, Faculty of Science and Education, University of Mostar, Matice hrvatske bb, 88000 Mostar, Bosnia and Herzegovina



**Abstract**

*N*-acetyl-L-cysteine (NAC) is a precursor of glutathione and a natural antioxidant. It is used to treat paracetamol overdose and to dissolve thick bronchial mucus. A novel, simple, reproducible, and rapid kinetic spectrophotometric method for determining NAC in pharmaceutical formulations is proposed. The method is based on a redox reaction where NAC reduces bis(bathocuproinedisulfonate)cuprate(II), forming a yellow-orange complex of bis(bathocuproinedisulfonate)cuprate(I). The resulting stable complex exhibited maximum absorbance at $\lambda = 483$ nm. Under optimised chemical reaction parameters, both the fixed-time and initial-rate methods were used to generate calibration curves. For the fixed-time method, the curve was linear in the concentration range of $3.0 \cdot 10^{-7}$ to $7.0 \cdot 10^{-5}$ mol l$^{-1}$, with the equation $y = 13140x - 0.0005$ and a coefficient of determination of $R^2 = 0.9999$. For the initial-rate method, the curve was linear in the concentration range of $7.0 \cdot 10^{-7}$ to $7.0 \cdot 10^{-5}$ mol l$^{-1}$ with the equation $y = 1.040x + 3.220$ and $R^2 = 0.9991$. The slope of the logarithmic form of the calibration curve (1.040) for the initial-rate method confirmed the pseudo-first order nature of the reaction. The proposed method was successfully applied for the determination of NAC in commercial pharmaceutical preparations, yielding results comparable to those obtained using the recommended method according to the pharmacopoeia.

**Keywords**

*Kinetics, spectrophotometry, N-acetyl-L-cysteine, bathocuproine disulfonate, redox reaction*

## 1 Introduction

The significance of the thiolic compound *N*-acetyl-L-cysteine (NAC) lies in its widespread applications and diverse pharmacological effects. NAC is used as a mucolytic to treat coughs by breaking disulphide bonds in mucus, thereby effectively reducing the formation of mucus secretions.[1] This compound is available commercially as a pharmaceutical product and dietary supplement due to its antioxidant and liver-protective effects. NAC enhances glutathione synthesis in the liver, a polypeptide crucial for detoxifying harmful substances. Its chelating properties also assist in the removal of heavy metals from the body.[2]

There is extensive[3–11] literature on the quantitative determination of this sulphur-containing compound in analytical and biological samples, as well as in pharmaceutical preparations. The British Pharmacopoeia specifies iodimetric redox titration for determining NAC.[12]

Spectrophotometry is the most widely used technique in the pharmaceutical analysis of NAC, and is also considered one of the most convenient techniques in pharmaceutical analysis due to its simplicity and accessibility. Kinetic spectrophotometric methods provide enhanced selectivity by measuring absorbance changes over reaction time, offering a significant advantage. With these methods, the possibility of interference with other active substances or excipients present in the pharmaceutical formulations is avoided, making them useful for routine quality control of pharmaceuticals.

Available literature indicates various methods employed for determining NAC in pharmaceutical preparations or in biological samples (tissue or cells), including: titrimetry,[13] spectrophotometry,[14] fluorimetry,[15] chromatography,[16,17] potentiometry,[18] conductometry[19] and voltammetry.[20,21]

Two kinetic methods with potentiometric detectors have also been developed. One involves the formation of sparingly soluble salts by reaction between the analyte and silver(I) ions[22] and the other involves the reaction between the analyte and iodate with iodide addition, both using commercially available selective iodide electrodes.[23] The kinetic methods for determining NAC using spectrophotometric detectors are listed in Table 1.

The aim of this study was to develop a novel, simple, rapid, and sensitive kinetic method for determining NAC in pharmaceutical preparations. This method is based on a single-step redox reaction where NAC reduces the bis(bathocuproinedisulfonate)cuprate(II) complex to form a stable orange-yellow bis(bathocuproinedisulfonate)cuprate(I) complex that absorbs at 483 nm.

* Corresponding author: Maja Biočić Šormaz, PhD
Email: maja.biocic@ktf-split.hr

*Table 1* – Analytical parameters of kinetic spectrophotometric methods reported in the literature and in the novel method for determining NAC in pharmaceutical formulations

*Tablica 1* – Analitički parametri kinetičkih spektrofotometrijskih metoda iz literature i nove metode za određivanje NAC-a u farmaceutskim pripravcima

| Ref. | Reagents | $\lambda$ / nm | Linear range / mol l$^{-1}$ | LOD / mol l$^{-1}$ | Kinetic method |
|---|---|---|---|---|---|
| 24 | Fe(III), 1,10-phenanathroline and Cu(II) | 510 | $8.0 \cdot 10^{-6} – 8.0 \cdot 10^{-5}$ | $2.0 \cdot 10^{-6}$ | Fixed-time method; (10 min) |
| 25 | 4-chloro-7-nitrobenzo-2-oxa 1,3-diazole (NBD-Cl) | 424 | $1.2 \cdot 10^{-5} – 1.3 \cdot 10^{-4}$<br>$2.45 \cdot 10^{-5} – 1.225 \cdot 10^{-4}$ | – | Fixed-time method; (30 min)<br>Initial-rate method |
| 26 | Fe(III) and 2,4,6-tripiridyl-*s*-triazine (TPTZ) | 593 | $1.0 \cdot 10^{-6} – 1.0 \cdot 10^{-4}$<br>$4.0 \cdot 10^{-6} – 1.0 \cdot 10^{-4}$ | $1.7 \cdot 10^{-7}$<br>$1.0 \cdot 10^{-6}$ | Fixed-time method; (5 min)<br>Initial-rate method |
| 27 | Cu(II) and neocuproine | 458 | $6.0 \cdot 10^{-7} – 8.0 \cdot 10^{-5}$ | $1.7 \cdot 10^{-7}$ | Fixed-time method; (1 min)<br>Initial-rate method |
| 28 | 2.2′-bipyridine (Bipy), $[Fe(CN)_6]^{4-}$ and $Hg^{2+}$ | 400 | $1.50 \cdot 10^{-6} – 5.35 \cdot 10^{-5}$ | – | Fixed-time method; (10 and 15 min) |
| Novel method | Cu(II) and bathocuproinedisulfonate disodium (BCS) | 483 | $3.0 \cdot 10^{-7} – 7.0 \cdot 10^{-5}$<br>$7.0 \cdot 10^{-7} – 7.0 \cdot 10^{-5}$ | $9.0 \cdot 10^{-8}$<br>$2.1 \cdot 10^{-7}$ | Fixed-time method; (1 min)<br>Initial-rate method |

LOD – limit of detection

## 2 Experimental

### 2.1 Chemical and reagents

All solutions used were prepared using analytical grade chemicals and deionised water, unless otherwise stated.

A precisely weighted amount (0.1632 g) of *N*-acetyl-L-cysteine (NAC, Acros Organics, New Jersey, USA) was quantitatively transferred into a 100 ml volumetric flask, and acetate-borate-phosphate buffer (pH = 2.0) was added to the mark. The prepared standard solution was stable for one month when stored at 4 °C, protected from sunlight. Working solutions of precisely determined concentrations were freshly prepared a few minutes prior to the analytical procedure by diluting with deionised water.

The acetate-borate-phosphate buffer solution with a concentration of 0.04 mol l$^{-1}$ and pH = 2 was prepared by adding 4.79 g of acetic acid (VWR Chemicals, France), 4.95 g of boric acid (Alkaloid, Skopje, Macedonia), and 5.45 g of phosphoric acid (Kemika, Zagreb, Croatia) in deionised water. After complete dissolution, the flask was filled with water to the 2.0 l mark. Buffer solutions with higher pH values were prepared by adding the required volume of a previously prepared sodium hydroxide solution, $c$(NaOH) = 2.0 mol l$^{-1}$. The change in pH value was monitored using a pH-meter (Mettler Toledo, Schwerzenbach, Switzerland).

A copper(II) solution with a concentration of 0.008 mol l$^{-1}$ was prepared by dissolving 99.9 mg of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, Kemika, Zagreb, Croatia) in a 50 ml flask with deionised water as the solvent.

A solution of the copper-affinity ligand bathocuproinedisulfonate (BCS) with a concentration of 0.002 mol l$^{-1}$ was prepared by dissolving 56.5 mg of bathocuproinedisulfonate disodium salt (Alfa Aesar, Karlsruhe, Germany) in a 50 ml flask with deionised water up to the mark.

In this research, two widely available medications were tested: Fluimukan 600 mg NAC (Sandoz d. o. o., Zagreb, Hrvatska) and Fluimukan 200 mg NAC (Sandoz d. o. o., Zagreb, Hrvatska). A precise amount of finely ground tablets from the commercial samples was transferred to a calibrated flask and dissolved and diluted in acetate-borate-phosphate buffer (pH = 2.0). No pretreatment of the samples was necessary, and the prepared solutions of the real samples were stable for 24 h. After adjusting the required concentration through appropriate dilution, the sample was injected into the prepared reagent solution 60 s after the start of absorbance measurement. To validate the method, a standard iodine solution was prepared following the Pharmacopoeia guidelines.[12]

### 2.2 Instrumentation and kinetic procedure

The kinetic measurements were conducted using a peristaltic pump with PTFE tubing, double beam UV-Vis spectrophotometer (Shimadzu UV-1601, Kyoto, Japan) equipped with a flow cell (Helma, Jamaica, New York), to detect absorbance at 483 nm corresponding to the concentration of bis(bathocuproinedisulfonate)cuprate(I) complex. The set-up of the kinetic manifold is detailed in earlier studies.[26,27]

Volumes of 0.45 ml of 0.008 mol l$^{-1}$ copper(II) solution, 1.80 ml of 0.002 mol l$^{-1}$ BCS solution, 10.0 ml of buff-

er pH = 3.0, and 1.75 ml deionised water were added in the reaction vessel with constant mixing. The reaction solution volume reached 15.0 ml after adding 1.0 ml of NAC into the reaction solution 60 s from the start of the experiment to initiate the reaction. The absorbance values were continuously monitored at 483 nm against reagent blank and recorded as a function of time. These values were subsequently used for calculations via the initial-rate and fixed-time methods. The output signals were recorded by connecting the spectrophotometer to a computer equipped with Shimadzu's Hyper UV-Vis software.

## 3 Results and discussion

The developed kinetic method for determining NAC using a spectrophotometric detector involves a direct, one-step approach where NAC reduces the bis(bathocuproinedisulfonate)cuprate(II) complex ((Eq. (1)). This produces a highly stable orange-coloured bis(bathocuproinedisulfonate)cuprate(I) complex with maximum absorbance at $\lambda = 483$ nm proportional to the NAC concentration.[29]

$$2RSH + 2[Cu(BCS)_2]^{2-} \rightleftarrows RSSR + 2[Cu(BCS)_2]^{3-} + 2H^+ \quad (1)$$

By analysing the kinetics of the proposed reaction, the parameters of the chemical reaction were optimised: pH, temperature, and the molar ratio of reagent components at constant concentration of NAC, $c(NAC) = 4 \cdot 10^{-5}$ mol l$^{-1}$.

### 3.1 Optimisation of the parameters

When investigating the influence of pH, the reaction rate remained practically unchanged in the pH range of 2.0 to 8.0, using an acetate-borate-phosphate buffer. The results showed no increase in signal or reaction rate at higher pH values (4.0 to 8.0). The highest absorbance was measured at a pH of 3.0, which was selected as the optimal condition for further optimisation. These findings suggest that NAC, a thiol compound, exhibits greater stability at lower pH values.

The temperature of the thermostated reaction vessel was varied from 20 to 50 °C to evaluate the effect of temperature on the reaction rate. No significant change in absorbance was observed across this wide temperature range. However, as the temperature of the reaction solution increased, the temperature of the tubes responsible for recirculating the reaction solution also increased, leading to the formation of air bubbles. The likelihood of air bubbles forming in the system increases with temperature. Thus, the influence of air bubbles in the system became more visible above 50 °C in the form of small irregularities in the recorded signals. The results indicated that the proposed redox reaction, which forms the basis for NAC determination, was essentially independent of temperature, and that the stability of the chemical reaction product remained consistent across the tested temperature range. At 25 °C, the formed complex demonstrated stability with a satisfactory reaction rate. As a result, 25 °C was selected as optimal temperature. Furthermore, the experiment can be performed without the need to thermostat the reaction mixture during the procedure.

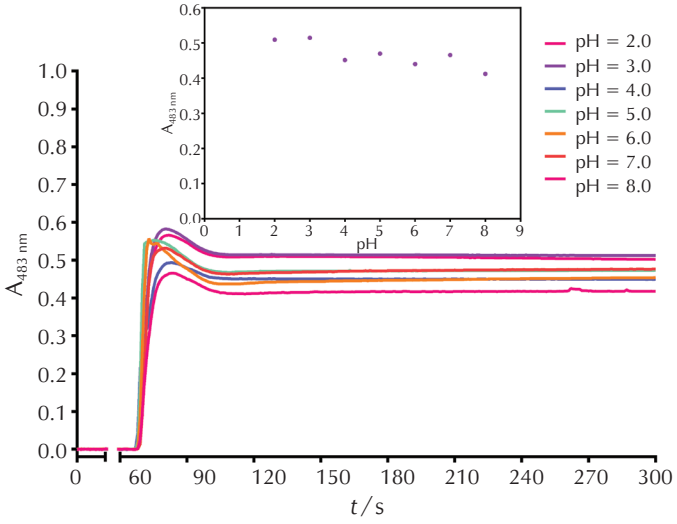


*Fig. 1* – Absorbance change as a function of time for the kinetic spectrophotometric determination of NAC at different pH values. Inset: Dependence of absorbance on pH.

*Slika 1* – Promjena apsorbancije kao funkcija vremena za kinetičko spektrofototometrijsko određivanje NAC-a pri različitim vrijednostima pH. Umetak: Apsorbancija kao funkcija vrijednosti pH.

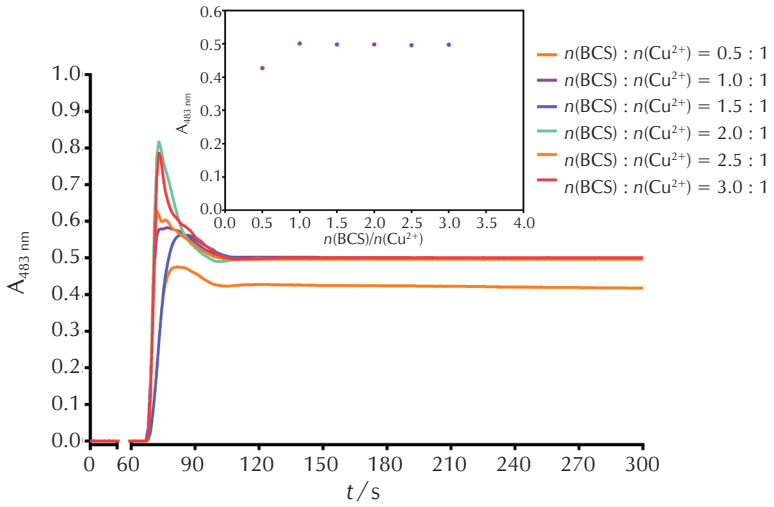


*Fig. 2* – Absorbance change as a function of time for kinetic spectrophotometric determination of NAC at different reagent molar ratios. Inset: Dependence of absorbance on reagent molar ratio.

*Slika 2* – Apsorbancija kao funkcija vremena za kinetičko spektrofototometrijsko određivanje NAC-a pri različitim molarnim omjerima reagensa. Umetak: Apsorbancija kao funkcija molnog omjera reagensa.

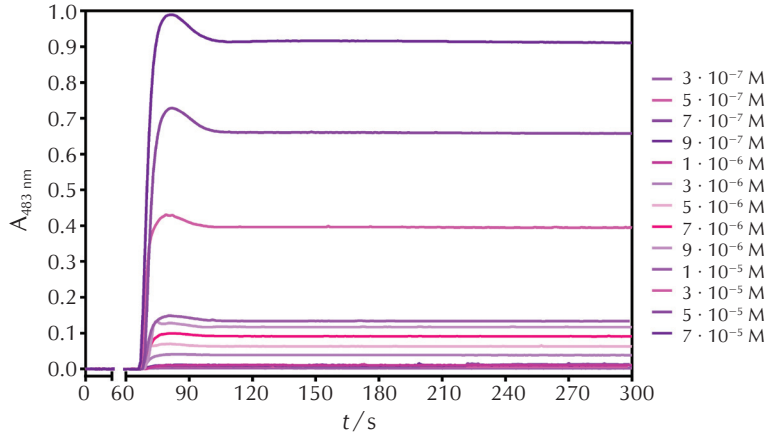


*Fig. 3* – Kinetic curves for the proposed redox reaction measured at optimum experimental conditions

*Slika 3* – Kinetičke krivulje predložene redoks-reakcije izmjerene pri optimalnim eksperimentalnim uvjetima

*Table 1* – Examined and optimised parameters of the chemical reaction for NAC determination

*Tablica 1* – Ispitani i optimizirani parametri kemijske reakcije za određivanje NAC-a

| Studied parameters | Examined values | Optimum conditions |
|---|---|---|
| pH | 2.0 – 8.0 | 3.0 |
| temperature / °C | 10 – 50 | 25 |
| $n$(BCS) : $n$(Cu²⁺) | 0.5 : 1 – 3.0 : 1 | 1 : 1 |

The molar ratio of the bis(bathocuproinedisulfonate)cuprate(II) complex was evaluated by maintaining the copper(II) concentration at $3.2 \cdot 10^{-4}$ mol l$^{-1}$, while varying the concentration of the BCS solution from $1.6 \cdot 10^{-4}$ to $9.6 \cdot 10^{-4}$ mol l$^{-1}$. Consequently, the molar ratio of BCS to copper(II) varied between 0.5 and 3.0. As the BCS concentration increased at a constant copper(II) concentration, the reaction rate and, consequently, the absorbance increased up to a ratio of 1 : 1, beyond which a constant value was reached. At higher ratios, no significant differences in the signal height were observed (Fig. 2).

### 3.2 Analytical characteristics of kinetic measurements

The calibration diagram was constructed under optimum conditions (Table 2) for varying concentrations of NAC, and the time-absorbance kinetics of these NAC concentrations were experimentally measured (Fig. 3). The experimental

*Table 3* – Results of regression analysis of the fixed-time method for determining NAC
*Tablica 3* – Rezultati regresijske analize kod metode određenog vremena za određivanje NAC-a

| Variable | 90 s | 120 s | 150 s | 180 s | 210 s | 240 s |
|---|---|---|---|---|---|---|
| Beer's Law / mol l$^{-1}$ | $3 \cdot 10^{-7} - 7 \cdot 10^{-5}$ | $3 \cdot 10^{-7} - 7 \cdot 10^{-5}$ | $3 \cdot 10^{-7} - 7 \cdot 10^{-5}$ | $3 \cdot 10^{-7} - 7 \cdot 10^{-5}$ | $3 \cdot 10^{-7} - 7 \cdot 10^{-5}$ | $3 \cdot 10^{-7} - 7 \cdot 10^{-5}$ |
| equation | $y = 13790x - 0.0008$ | $y = 13140x - 0.0005$ | $y = 13150x - 0.0004$ | $y = 13140x - 0.0006$ | $y = 13140x - 0.0009$ | $y = 13110x - 0.0003$ |
| coefficient of determination | 0.9997 | 0.9999 | 0.9999 | 0.9999 | 1.0000 | 0.9999 |
| LOD / mol l$^{-1}$ | $9 \cdot 10^{-8}$ | $9 \cdot 10^{-8}$ | $9 \cdot 10^{-8}$ | $9 \cdot 10^{-8}$ | $9 \cdot 10^{-8}$ | $9 \cdot 10^{-8}$ |

data obtained *via* the kinetic manifold were analysed by two computational methods: the fixed-time method and the initial-rate method, using Excel and GraphPad software.

The mathematical method of least squares was applied for regression analysis, including the calculation of the linearity equation with the corresponding coefficient of determination ($R^2$) for both computational methods (Table 4). Based on the optimised chemical conditions of the proposed redox reaction, the kinetic curves confirmed the kinetics of redox reactions to be rapid.

### *3.2.1 Fixed-time method*

In this method, the analytical signals for various NAC concentrations were recorded at a specific time during the kinetic measurement. The calibration curves depicting the dependence of absorbance on NAC concentration were constructed at predefined time points. The time point was selected to achieve the best linearity and the widest linear concentration range. Acceptable results were observed at the start of the chemical reaction, with satisfactory linearity achieved as early as 120 s (1 min after the reaction began (Table 3)).

### *3.2.2 Initial-rate method*

Using this method, the initial reaction rate ($K$) was determined from the slope of the tangent taken from the steep part of the kinetic curves ($\Delta A/\Delta t$) when the reaction order $n$ equalled one (Eq. (2)). The logarithmic form of the reaction rate equation ($\log K$) is expressed as a logarithmic function of NAC concentration ($\log c$) according to the Eq. (3).

$$K = \frac{\Delta A}{\Delta t} = k' \cdot c^n \tag{2}$$

$$\log K = \log \frac{\Delta A}{\Delta t} = \log k' + n \log c \tag{3}$$

$K$ is the reaction rate, $A$ is the absorbance, $t$ is the measured time, $k$ is the constant of the reaction rate for the pseudo-first order reaction, and $c$ is the molar concentration of the analyte (NAC).

According to the logarithmic equation of the straight line, the slope $n$ was calculated from the experimental data, which was approximately equal to 1 for NAC ($n = 1.040$). This confirmed that the proposed redox reaction between NAC and the bis(bathocuproinedisulfonate)cuprate(II) complex followed pseudo-first order kinetics (Fig. 5).

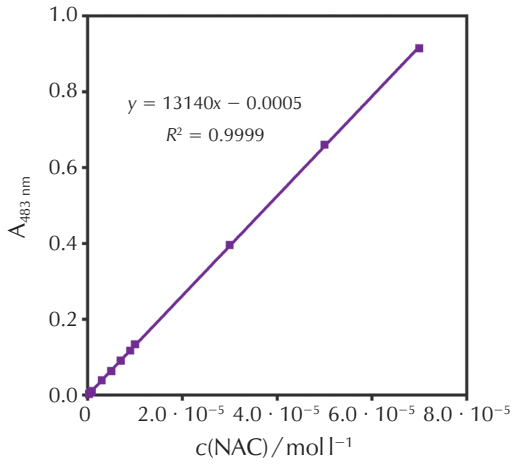


*Fig. 4* – Fixed-time method calibration curve (120 s)
*Slika 4* – Krivulja umjeravanja za metodu određenog vremena (120 s)

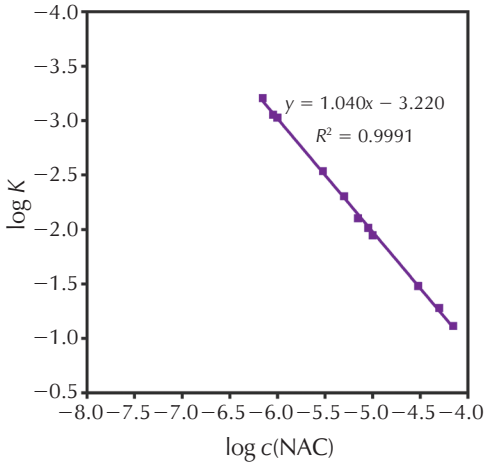


*Fig. 5* – Initial-rate method calibration curve
*Slika 5* – Krivulja umjeravanja za metodu početne brzine

*Table 4* – Analytical characteristics of the fixed-time and initial-rate methods
*Tablica 4* – Analitičke karakteristike metode određenog vremena i metode početne brzine

| Parameters studied | Fixed-time method | Initial-rate method |
|---|---|---|
| Beer's Law / mol l$^{-1}$ | $3.0 \cdot 10^{-7} - 7.0 \cdot 10^{-5}$ | $7.0 \cdot 10^{-7} - 7.0 \cdot 10^{-5}$ |
| linear equation | $y = 13140x - 0.0005$ | $y = 1.040x - 3.220$ |
| coefficient of determination | 0.9999 | 0.9991 |
| limit of detection / mol l$^{-1}$ | $9.0 \cdot 10^{-8}$ | $2.1 \cdot 10^{-7}$ |

*Table 5* – Study of potential interferences
*Tablica 5* – Utjecaj mogućih interferirajućih tvari

| Substance | Tolerable concentration / mol l$^{-1}$ | Relative error / % |
|---|---|---|
| Glucose | $2.0 \cdot 10^{-2}$ | +3.83 |
| Fructose | $2.0 \cdot 10^{-2}$ | +1.92 |
| Lactose | $2.0 \cdot 10^{-3}$ | +4.89 |
| $Na_2SO_4$ | $2.0 \cdot 10^{-3}$ | +0.11 |
| $KNO_3$ | $2.0 \cdot 10^{-2}$ | +1.83 |
| Citric acid | $2.0 \cdot 10^{-3}$ | −1.01 |
| Tartaric acid | $2.0 \cdot 10^{-3}$ | −1.56 |
| Sodium citrate | $2.0 \cdot 10^{-3}$ | +0.88 |

### 3.3 Study of interferences

The potential interference of excipients in the mixture with NAC could cause errors in the determination of NAC. Since such excipients are commonly present in commercial pharmaceutical formulations, the influence of foreign ions or substances was investigated by measuring the NAC concentration ($c = 4.0 \cdot 10^{-5}$ mol l$^{-1}$) in mixed solutions containing varying concentrations of these potential interferents (500, 200, 100 or 50 molar excess). Table 5 presents the highest permissible concentrations of the interfering species, i.e., the highest allowed ratio of the interferent to analyte ($E_r = \pm 5$ %).

Glucose, fructose, and $KNO_3$ did not produce errors greater than 5 % in the determination of $4.0 \cdot 10^{-5}$ mol l$^{-1}$ NAC at molar excesses of up to 500. The signal with tolerance limit was measured even with 50 times the amount of lactose, citric acid, tartaric acid, sodium citrate, and sodium sulphate relative to NAC (Table 5). It is worth noting that the tested concentrations of interfering species were significantly higher than those commonly found in commercial pharmaceuticals.

### 3.4. Evaluation of accuracy

The accuracy of the method was assessed by evaluating the recovery of kinetic spectrophotometric methods for determining NAC processed through both the fixed-time and initial-rate methods (Table 6). The recovery of the proposed kinetic spectrophotometric method was tested by adding known amounts of NAC standard solution to a pharmaceutical sample solution prior to analysis using a developed method. The recovery results for the developed kinetic method ranged between 96.2 % and 101.1 % for the fixed-time method, and between 92.5 % and 104.5 % for the initial-rate method. These findings demonstrate the acceptable accuracy of the developed kinetic method, and confirm the absence of interfering substances in the samples used.

### 3.5 Application in pharmaceutical preparations

The quantitative determination of NAC in two different commercially available pharmaceutical formulations was

*Table 6* – Evaluation of the accuracy of the proposed kinetic spectrophotometric methods determining NAC using the fixed-time method and the initial-rate method
*Tablica 6* – Ispitivanje točnosti predložene kinetičke spektrofotometrijske metode za određivanje NAC-a metodom određenog vremena i metodom početne brzine

| Sample | Added / mg | Fixed-time method | | Initial-rate method | |
|---|---|---|---|---|---|
| | | Found ± SD[(b)] / mg | Recovery / % | Found ± SD[(b)] / mg | Recovery / % |
| Fluimukan 600 mg [(a)] | 0 | 596.4 ± 0.4 | – | 594.1 ± 0.7 | – |
| | 50 | 652.0 ± 0.5 | 100.3 | 660.4 ± 1.1 | 101.6 |
| | 100 | 704.9 ± 0.4 | 100.7 | 647.4 ± 0.9 | 92.5 |
| | 150 | 758.3 ± 1.2 | 101.1 | 783.8 ± 1.4 | 104.5 |
| | 200 | 769.6 ± 1.8 | 96.2 | 808.0 ± 1.3 | 101.0 |

[(a)] Effervescent NAC 600 mg tablets with excipients.
[(b)] Standard deviation (SD) of three determinations *per* sample.

carried out using the proposed kinetic spectrophotometric method. For this purpose, the average mass of one tablet was dissolved in deionised water from the ground contents of 5 tablets, and diluted in a Britton-Robinson buffer solution (pH = 2.0). The sample solution was then appropriately diluted to bring the pharmaceutical concentration within the calibration range. Three measurements were performed for each sample, and the average absorbance value was used for further calculations using both computational kinetic methods.

*Table 7* – Comparison of the developed method with the standard method[12]

*Tablica 7* – Usporedba razvijene metode sa standardnom metodom[12]

| Sample | Fixed-time method[(c)] / mg | Initial-rate method[(c)] / mg | Standard method[(c)] / mg |
|---|---|---|---|
| Fluimukan 600 mg[(a)] | 584.7 ± 1.3 | 592.8 ± 0.8 | 622.0 ± 0.6 |
| Fluimukan 200 mg[(b)] | 205.4 ± 0.9 | 212.0 ± 1.2 | 201.9 ± 1.1 |

[(a)] Effervescent NAC 600 mg tablets with excipients.
[(a)] Effervescent NAC 200 mg tablets with excipients.
[(c)] Found ± standard deviation (SD) of three determinations *per* sample.

The experimental values from both computational methods showed a good correlation between the proposed kinetic spectrophotometric methods and the redox titrations for the determination of NAC as outlined in the Pharmacopoeia (Table 7). This developed kinetic method is highly reliable for determining NAC in pharmaceutical formulations without the need for prior treatment of the real sample.

## 4 Conclusions

This study describes the development and full validation of a novel kinetic spectrophotometric method for determining NAC in pharmaceutical formulations. The final stable reaction product, bis(bathocuproinedisulfonate)cuprate(I) was formed by the redox reaction between NAC and the reagent bis(bathocuproinedisulfonate)cuprate(II) complex. Both the initial-rate method and the fixed-time method were applied, and were sensitive enough to analyse small amounts of NAC. Compared with previously developed methods reported in the literature, the newly developed kinetic spectrophotometric method for NAC determination demonstrated a wider linear dynamic range for both computational methods (spanning two or more orders of magnitude) and greater sensitivity with lower detection limits. The recovery results including both methods (92.5–104.5 %) showed satisfactory accuracy and reproducibility of the proposed method for quantifying NAC in pharmaceutical formulations. Excipients typically present in pharmaceutical preparations did not interfere with the developed method. The successfully applied kinetic method for NAC determination in pharmaceutical preparations showed a good agreement of the kinetic results with the iodimetric method prescribed in the Pharmacopoeia. Therefore, this study presents a more sensitive, faster, and simpler method for determining NAC in both pure and pharmaceutical preparations, based on an uncatalysed, fast redox reaction that requires no derivatisation or prior sample treatment.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Science and Education, Republic of Croatia, through the institutional financing of researcher Lea Kukoc Modun.

### List of abbreviations

BCS – bathocuproine disulfonate disodium
$Cu^{2+}$ – copper(II)
$[Cu(BCS)_2]^{2-}$ – bis(bathocuproinedisulfonate)cuprate(II) complex
$[Cu(BCS)_2]^{3-}$ – bis(bathocuproinedisulfonate)cuprate(I) complex
$E_r$ – relative error
LOD – limit of detection
LOQ – limit of quantification
NAC – *N*-acetyl-L-cysteine
PTFE – polytetrafluoroethylene
$R^2$ – coefficient of determination
RSH – thiol compound

### References
### Literatura

1. *G. S. Kelly*, Clinical applications of *N*-acetylcysteine, Altern. Med. Rev. **3** (1998) 114–127.
2. *G. K. Schwalfenberg*, *N*-Acetylcysteine: A Review of Clinical Usefulness (an Old Drug with New Tricks), J. Nut. Metabol. **2021** (2021) 1–13, doi: https://doi.org/10.1155/2021/9949453.
3. *B. Abt, A. Hartmann, A. Pasquarelli, S. Strehle, B. Mizaikoff, C. Kranz*, Electrochemical Determination of Sulphur-containing Pharmaceuticals Using Boron-doped Diamond Electrodes, Electroanalysis **28** (2016) 1641–1646, doi: https://doi.org/10.1002/elan.201501150.
4. *N. Y. Bondarenko, M. Y. Blazheyevskiy*, Determination of *N*-acetylcysteine in tablets by means of chemiluminescence inhibition method, Methods Obj. Chem. Anal. **13** (3) (2018) 110–114, doi: https://doi.org/10.17721/MOCA.2018.110-114.
5. *M. M. Foroughi, H. Beitollahi, S. Tajik, A. Akbari, R. Hosseinzadeh*, Electrochemical determination of *N*-acetylcysteine and folic acid in pharmaceutical and biological samples using a modified carbon nanotube paste electrode, Int. J. Electrochem. Sci. **9** (2014) 8407–8421, doi: https://doi.org/10.1016/s1452-3981(23)11056-x.
6. *G. Özen, E. Nemutlu*, Green HPLC method for simultaneous determination of *N*-acetylcysteine and L-ascorbic acid in co-formulated pharmaceutical products, J. Res. Pharmacol. **28** (2024) 1777–1790, doi: https://doi.org/10.29228/jrp.850.

7. *A. Elmekawy*, Simultaneous Determination of Residual Palladium and Thiol Homogeneous Scavenger *N*-Acetylcysteine in Active Pharmaceutical Ingredients Using Inductive Coupled Plasma-Mass Spectrometry, Org. Process Res. Develop. **25** (2021) 1352–1359, doi: https://doi.org/10.1021/acs.oprd.0c00542.
8. *R. Gatti*, Simultaneous Determination of Taurine, *N*-Acetylcysteine, Glycine and Methionine in Commercial Formulations by High-Performance Liquid Chromatography, Chromathographia **82** (2019) 1833–1837, doi: https://doi.org/10.1007/s10337-019-03808-8.
9. *S. Meenakshi, G. Kaladevi, K. Pandian, P. Wilson*, Cobalt phthalocyanine tagged graphene nanoflakes for enhanced electrocatalytic detection of *N*-acetylcysteine by amperometry method, Ionics **24** (2018) 2807–2819, doi: https://doi.org/10.1007/s11581-017-2410-5.
10. *A. Roydeva, A. Milanova*, LC–MS/MS determination of *N*-acetyl-L-cysteine in chicken plasma, Biomed. Chromatographia **38** (2024) e5854, doi: https://doi.org/10.1002/bmc.5854.
11. *A. Kamińska, P. Olejarz, K. Borowczyk, R. Głowacki, G. Chwatko*, Simultaneous determination of total homocysteine, cysteine, glutathione, and *N*-acetylcysteine in brain homogenates by HPLC, J. Sep. Sci. **41** (2018) 3241–3249, doi: https://doi.org/10.1002/jssc.201800381.
12. *British Pharmacopoeia*, Acetylcysteine, The Stationery Office London, 2018, 109–113.
13. *M. S. Rizk, F. M. Belal, M. Eid*, 2-Iodoxybenzoate as a titrant for the determination of some pharmaceutically-important thiol compounds, Acta Pharm. Hung. **63** (1993) 13–18.
14. *F. Buhl, M. Galkowska*, Spectrophotometric determination of *N*-acetyl-L-cysteine using leucoxylenecyanol FF, Chem. Anal. -Warsaw **51** (2006) 623–629.
15. *S. M. Al-Ghannam, A. M. El-Brashy, B. S. Al-Farhan*, Fluorimetric determination of some thiol compounds in their dosage forms, Farmaco **57** (2002) 625–629, doi: https://doi.org/10.1016/S0014-827X(02)01223-5.
16. *D. Harada, S. Naito, Y. Kawauchi, K. Ishikawa, O. Koshitani, I. Hiraoka, M. Otagiri*, Determination of Reduced, Protein-Unbound, and Total Concentrations of *N*-Acetyl-L-cysteine and L-Cysteine in Rat Plasma by Postcolumn Ligand Substitution High-Performance Liquid Chromatography, Anal. Biochem. **290** (2001) 251–259, doi: https://doi.org/10.1006/abio.2000.4980.
17. *B. Toussaint, C. Pitti, B. Streel, A. Ceccato, P. Hubert, J. Crommen*, Quantitative analysis of *N*-acetylcysteine and its pharmacopeial impurities in a pharmaceutical formulation by liquid chromatography-UV detection-mass spectrometry, J. Chromatogr. A **896** (2000) 191–199, doi: https://doi.org/10.1016/S0021-9673(00)00741-X.
18. *M. Kolar, D. Dobcnik*, Chemically prepared silver electrode for determination of *N*-acetyl-L-cysteine by flow-injection potentiometry, Pharmazie **58** (2003) 25–28.
19. *B. C. Janegitz, W. T. Suarez, O. Fatibello-Filho, L. H. Marcolino-Junior*, Conductometric Determination of *N*-acetylcysteine in Pharmaceutical Formulations Using Copper(II) Sulphate as Titrant, Anal. Lett. **41** (2008), 3264–3271, doi: https://doi.org/10.1080/00032710802507554.
20. *H. R. Zare, F. Chatraei*, Preparation and electrochemical characteristics of electrodeposited acetaminophen on ruthenium oxide nanoparticles and its role as a sensor for simultaneous determination of ascorbic acid, dopamine and *N*-acetyl-L-cysteine, Sens. Actuators B Chem. **160** (2011) 1450–1457, doi: https://doi.org/10.1016/j.snb.2011.10.012.
21. *A. A. Ensafi, H. Karimi-Maleh, S. Mallakpour, M. Hatami*, Simultaneous determination of N-acetylcysteine and acetaminophen by voltammetric method using *N*-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide modified multiwall carbon nanotubes paste electrode, Sens. Actuators B Chem. **155** (2011) 464–472, doi: https://doi.org/10.1016/j.snb.2010.12.048.
22. *A. Martinović, Nj. Radić*, Kinetic Potentiometric Determination of some Thiols with Iodide Ion-Sensitive Electrode, Anal. Lett. **40** (2007) 2851–2859, doi: https://doi.org/10.1080/00032710701603884.
23. *A. Martinović, Nj. Radić*, Kinetic Potentiometric Determination of Penicillamine and *N*-Acetyl-L-Cysteine Based on Reaction with Iodate, Acta Chim. Slov. **56** (2009) 503–506.
24. *A. Martinović, L. Kukoč-Modun, Nj. Radić*, Kinetic spectrophotometric determination of thiols and ascorbic acid, Anal. Lett. **40** (2007) 805–815, doi: https://doi.org/10.1080/00032710601017938.
25. *E. A. Taha, N. Y. Hassan, F. A. Aal, L. E.-S. A. Fattah*, Kinetic Spectrophotometic Determination of Acetylcysteine and Carbocisteine in Bulk Powder and in Drug Formulations, Sci. Asia **34** (2008) 107–113, doi: http://dx.doi.org/10.2306/scienceasia1513-1874.2008.34.107.
26. *L. Kukoc-Modun, Nj. Radic*, Kinetic spectrophotometric determination of *N*-acetyl-L-cysteine based on a coupled redox-complexation reaction, Anal. Sci. **26** (2010) 491–495, doi: https://doi.org/10.2116/analsci.26.491.
27. *Nj. Radić, L. Kukoc-Modun, M. Biocic*, Kinetic Spectrophotometric Determination of *N*-acetyl-L-cysteine based on the reduction of copper(II)-neocuproine reagent, Croat. Chem. Acta **86** (2013) 65–71, doi: https://doi.org/10.5562/cca2161.
28. *B. Lal, A. Asthana, V. K. Singh, K. Srivastava*, Quantification of *N*-acetylcysteine in drug formulations using inhibitory kinetic spectrophotometric method, J. Res. Pharm. **26** (2022) 1713–1722, doi: https://doi.org/10.29228/jrp.262.
29. *L. Kukoč-Modun, D. Tsikas, T. Kraljević, M. Biočić, Nj. Radić*, Kinetic spectrophotometric determination of *N*-acetyl-L-cysteine ethyl ester (NACET) generating chromogenic copper (I) L n complexes with different ligands, Croat. Chem. Acta **90** (2017) 263–271, doi: https://doi.org/10.5562/cca3135.

## SAŽETAK

# Razvoj i validacija nove kinetičke spektrofotometrijske metode za određivanje *N*-acetyl-L-cisteina

*Maja Biočić Šormaz*,[a*] *Tomislav Kraljević* [b] i *Lea Kukoč-Modun* [a]

*N*-acetil-L-cistein (NAC) je prekursor glutationa i prirodni je antioksidans. Upotrebljava se za liječenje predoziranja paracetamolom i za otapanje guste bronhijalne sluzi. Predložena je nova, jednostavna, ponovljiva i brza kinetička spektrofotometrijska metoda za određivanje NAC-a u farmaceutskim pripravcima. U redoks reakciji odvija se redukcija bis(batokuproindisulfonato)kuprat(II) s NAC-om, pri čemu nastaje žuto-narančasti kompleks bis(batokuproindisulfonato)kuprat(I), na čijoj se reakciji temelji metoda. Rezultirajući stabilni kompleks pokazuje maksimalnu apsorbanciju pri $\lambda = 483$ nm. Pri optimiziranim parametrima kemijske reakcije, metode određenog vremena i početne brzine primijenjene su za konstruiranje krivulje umjeravanja. Krivulja umjeravanja je pokazala linearnost u rasponu koncentracija od $3{,}0 \cdot 10^{-7}$ do $7{,}0 \cdot 10^{-5}$ mol l$^{-1}$ kod metode određenog vremena uz pripadajuću jednadžbu linearnosti $y = 13140x - 0{,}0005$ i koeficijent determinacije $R^2 = 0{,}9999$. Za primijenjenu metodu početne brzine krivulja je pokazala linearni raspon koncentracija od $7{,}0 \cdot 10^{-7}$ do $7{,}0 \cdot 10^{-5}$ mol l$^{-1}$ uz jednadžbu linearnosti $y = 1{,}040x + 3{,}220$ i koeficijent determinacije $R^2 = 0{,}9991$. Reakcija je prvog reda, što je potvrđeno nagibom krivulje umjeravanja (1,040) kod metode početne brzine. Predložena metoda uspješno je primijenjena za određivanje NAC-a u komercijalnim farmaceutskim pripravcima. Rezultati dobiveni predloženom metodom usporedivi su s rezultatima dobivenim metodom koju preporučuje farmakopeja.

**Ključne riječi**
*Kinetika, spektrofotometrija, N-acetil-L-cistein, batokuproin disulfonat, redoks-reakcija*

[a] *Sveučilište u Splitu, Kemijsko-tehnološki fakultet, Zavod za analitičku kemiju i kemiju okoliša, Ruđera Boškovića 35, 21 000 Split*
[b] *Sveučilište u Mostaru, Fakultet prirodoslovno-matematičkih i odgojnih znanosti, Zavod za kemiju, Matice hrvatske bb, 88 000 Mostar, Bosna i Hercegovina*

Izvorni znanstveni rad
*Prispjelo 23. listopada 2024.*
*Prihvaćeno 5. prosinca 2024.*