Kinetic Spectrophotometric Determination of N-acetyl-L-cysteine Based on the Reduction of Copper(II)-neocuproine Reagent†

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Abstract. A novel simple kinetic spectrophotometric method for the determination of N-acetyl-L-cysteine (NAC) has been developed and validated. The proposed method is based on the reduction of Cu²⁺-neocuproine reagent to Cu⁺-neocuproine with the analyte, in a Britton-Robinson buffer solution (pH = 3.0). The non-steady state absorbance of the formed Cu⁺-neocuproine complex is measured at 458 nm. The initial rate and fixed time (at 1 min) methods were utilized for constructing the calibration graphs. The calibration curves for both methods were linear in concentration range from 6.0 × 10⁻⁷ to 8.0 × 10⁻⁵ mol L⁻¹ with the limit of detection 1.7 × 10⁻⁷ mol L⁻¹. The slope of the initial method calibration curve (1.0181) confirmed the first order reaction. Both proposed methods were successfully applied for the determination of NAC in its commercial pharmaceutical formulations. (doi: 10.5562/cca2161)

Keywords: N-acetyl-L-cysteine, Kinetic spectrophotometry, Initial rate method, Fixed time method, Pharmaceutical analysis

INTRODUCTION

N-acetyl-L-cysteine (acetylcysteine, NAC) is a synthetic aminothiol antioxidant used in medicine as a mucolytic agent with the aim of reducing the viscosity of pulmonary secretions in a variety of respiratory illness. Intravenous administration of NAC is used extensively in the management of paracetamol (acetaminophen) poisoning. Further more, NAC is a physiological substance.¹ The European Pharmacopeia recommended iodimetric titration for pharmaceutical formulations analysis.⁷ Other reported methods described in the literature comprise: titrimetry,³,⁴ spectrophotometry,⁵,⁹ fluorimetry,¹⁰⁻¹² chromatography,¹³,¹⁴ potentiometry,¹⁵ conductometry¹⁶ and voltammetry¹⁷⁻¹⁹ for the determination of NAC in pure form, in dosage forms and in biological samples.

However, these methods are not sufficiently sensitive and selective, or some of them require expensive instrumentation and are too expensive for routine analysis.

Kinetic methods are a powerful tool for drug analysis as they permit sensitive and selective determination using affordable detectors within a few minutes with no sample pre-treatment in many cases. In fact, by using kinetic method determination of an analyte in a mixture may be possible when analyte and other species in mixture have sufficient differences of reaction rates. In our laboratory we research kinetic methods of analysis by using potentiometric and spectrophotometric detectors, almost forty years. In the first published kinetic analytical method a commercial fluoride ion-selective electrode as potentiometric sensor was used.²⁰ Our research team has published two kinetic potentiometric methods for the NAC determination.²¹,²² In addition, spectrophotometric technique is the most widely used in pharmaceutical analysis, due to its inherent simplicity, economic advantage, and wide availability in the most quality control laboratories. The application of kinetic spectrophotometric methods offers some specific advantages over classical spectrophotometry, such as improved selectivity due to the measurement of the evolution of the analytical signal (absorbance) with the reaction time. The literature is still poor in analytical procedures based on kinetic spectrophotometry for the determination of the examined analyte. Beside two kinetic spectrophotometric methods for the determination of NAC developed by our team,²³,²⁴ there are only two published kinetic spectrophotometric methods.²⁵,²⁶

In this report a novel simple and sensitive kinetic spectrophotometric method with the chromogenic reagent, copper(II)-neocuproine for the determination of...
NAC is described and validated. Ease of the application, sensitivity, short analysis time, low cost and reliability are its main advantages. The initial rate and fixed time methods, after their optimization and validation, are adopted for the determination of NAC in its pharmaceutical formulations.

**EXPERIMENTAL**

**Materials**

All chemicals were of analytical-reagent grade and solutions were prepared in deionised water (Milli Q, Millipore, Saint Quentin, Yvelines, France).

A stock solution of NAC \(1.0 \times 10^{-2}\) mol L\(^{-1}\) was prepared by dissolving 13.2 mg of NAC (Merck, Darmstadt, Germany) in Britton-Robinson buffer solution (pH = 3.0) up to 100.0 mL in volume and stored at 4 °C. Working solutions of NAC were prepared daily by dilution of the stock solution with deionised water.

A stock solution of Cu\(^{II}\) \(1.0 \times 10^{-2}\) mol L\(^{-1}\) was prepared by dissolving 250.0 mg of copper sulphate pentahydrate (Kemiya, Zagreb, Croatia) in a 100.0 mL volumetric flask, and about 90.0 mL of deionised water was added. The closed volumetric flask with the content was treated with sonication for about 15 minutes. Copper (II)-neocuproine reagent was prepared as follows: 25.0 mg of copper sulphate pentahydrate (Kemiya, Zagreb, Croatia) was transferred to a 100.0 mL volumetric flask, and about 90.0 mL of deionised water was added. Solubility of neocuproine is improved in the mixture with the Cu\(^{II}\) because the complex \([\text{Cu(neocuproine)}]^{2+}\) is formed.\(^{27}\) The molar ratio of neocuproine and Cu\(^{II}\) in the reaction mixture was determined in the optimization part of the experiment. According to these results the copper(II)-neocuproine reagent was prepared as follows: 25.0 mg of copper sulphate pentahydrate and 50.0 mg of neocuproine were dissolved in the 100.0 mL of deionised water. The molar ratio of neocuproine: Cu\(^{II}\) was 2.4:1. The dissolving process was enhanced by sonication for about 15 minutes. Copper (II)-neocuproine reagent was stable for at least 30 days stored at 4 °C. Britton-Robinson buffer solution (Acetic/Boric/Phosphoric acid buffer solution) was prepared by mixing acetic, boric and phosphoric acids of final concentrations 4 \(\times 10^{-2}\) mol L\(^{-1}\) (pH is about 2) and adjusting the pH value by adding sodium hydroxide solution, \(c = 2.0\) mol L\(^{-1}\). Adjustments and measurements of pH were carried out with a Mettler Toledo SevenMulti potentiometer (Mettler Toledo, Schwerzenbach, Switzerland) equipped with combined glass electrode Mettler Toledo InLab\(^{413}\). For the validation part of the experiments an iodine solution, 0.5 mol L\(^{-1}\), was prepared and standardized according to the literature.\(^{2}\)

**Sample Preparation**

The commercial pharmaceutical formulations analysed by proposed kinetic spectrophotometric method were granules, Fluimukan 100 mg, syrup, Fluimukan Junior and dispersible tablets, Fluimukan Akut 600 mg, all produced by Lek d.d., Ljubljana, Slovenia. The solutions of pharmaceutical products were prepared by dissolving suitable amounts of the commercial samples in Britton-Robinson buffer solution (pH = 3.0) and diluting the resulting solution, with the deionised water, to adjust the concentration required by the adopted experimental conditions.

For the validation part of the experiments an iodine solution, 0.5 mol L\(^{-1}\), was prepared and standardized according to the literature.\(^{2}\)

**Instrumentation**

The kinetic manifold for spectrophotometric determination consisted of an Ismatec IPC eight-channel peristaltic pump (Ismatec, Zurich, Switzerland) and Shimadzu UV-1601 (Shimadzu, Kyoto, Japan) UV-Vis spectrophotometer equipped with a Hellma (Jamaica, NY) flow cell of 160 μL internal volume and 10 mm optical path. The spectrometric instrument was set at 458 nm for all absorbance measurements. The kinetic manifold has been described in more details previously.\(^{24}\)

**Procedure**

For the determination of NAC, a mixture of reagents was prepared as follows. In a reaction vessel with 6.0 mL of Britton-Robinson buffer solution (pH = 3.0), 2.5 mL of copper(II)-neocuproine reagent and 15.5 mL of deionized water were added. In this reagent solution, 1 min after beginning of a measurement, a 1.0 mL portion of the analyte or sample was added to start the reaction. The final volume of the reaction solution in the thermostated vessel (25 °C) was 25.0 mL. At a flow rate of 6 mL min\(^{-1}\) and \(\lambda = 458\) nm, the absorbance of the produced \([\text{Cu(neocuproine)}]^{2+}\) complex was continuously recorded as the function of time. The frequency of data recording was 1 s\(^{-1}\).

**Data Acquisition and Processing**

The recorded kinetic data were transformed to a GraphPad Prism Ver. 4.03 for Windows (GraphPad Software, San Diego, CA) for curve fitting, regression analysis and statistical calculations. The initial rate and fixed time methods were utilized for constructing the calibration graphs. Initial reaction rates (K) were determined from the slopes of the absorbance-time curves. The logarithms of the reaction rates were plotted as a function of logarithms of NAC concentrations. The graph of the fixed time method was constructed by

plotting the absorbance measured at a fixed time versus \(c\) (NAC). A fixed time interval of 1 min was selected since linearity was additionally improved.

**RESULTS AND DISCUSSION**

The proposed method is based on the redox reaction (Equation (1)) in which NAC (RSH compound) reduces \(\text{Cu}^{II}\)-neocuproine complex to orange-yellow \(\text{Cu}^{I}\)-neocuproine complex:

\[
2\text{RSH} + 2[\text{Cu}(\text{neocuproine})_2]^{2+} \rightarrow \text{RSSR} + 2[\text{Cu}(\text{neocuproine})_2]^{+} + 2\text{H}^+ \quad (1)
\]

The spectrum of \(\text{Cu}^{I}\)-neocuproine complex (reference cell: deionised water) and \(\text{Cu}^{II}\)-neocuproine complex are shown in the Figure 1 curve (a) and (b), respectively.

The proposed redox reaction is very fast. However the coloured product of the reaction is rather unstable probably due to oxidation of \(\text{Cu}^{I}\) with the oxygen from the air (Figure 2). Therefore it is very important to measure the signal in the kinetic part of the reaction i.e. under dynamic conditions in which the concentrations of reactants and products are changing as a function of time.

**Optimization of the Reaction Conditions**

The effect of the pH was investigated over the range 2.0 – 8.0 using Britton-Robinson buffer solution. The appropriate pH values were adjusted by adding sodium hydroxide solution, \(c = 2.0\) mol L\(^{-1}\).

Although the reaction rates and the absorbances did not significantly change for the broad scale of the pH values in the first minute of the reaction, the signal was more stable at lower pH values. Besides, NAC is more stable at lower pH values. Therefore, a buffered reaction medium of pH = 3.0 was chosen for the further measuring.

Molar ratio of \(\text{Cu}^{II}\) and neocuproine in the reaction mixture was investigated. During the optimization experiment, the concentration of \(\text{Cu}^{II}\) maintained constant, \((1.0 \times 10^{-4}\) mol L\(^{-1}\)), and the concentration of neocuproine was changed in range: \(1.0 \times 10^{-4}\) mol L\(^{-1}\) to \(3.5 \times 10^{-4}\) mol L\(^{-1}\). Accordingly, the molar ratio of neocuproine / \(\text{Cu}^{II}\) has changed from 1.0 to 3.5. The results show that by increasing the neocuproine concentration, the reaction rate and the absorbance increased and reached a constant value when molar ratio of neocuproine / \(\text{Cu}^{II}\) was 2.4.

The effect of the reaction temperature on the signal intensity was examined by varying the temperature of the thermostated reaction vessel in range from 20 to 40°C.

The results showed that the proposed redox reaction is temperature independent in this range. The rate of the reaction and the stability of the signal remained the same in the wide temperature range. The reproducibility of measuring the same concentration of analyte at different temperatures was satisfying and the relative standard deviation was lower then 0.5%. Therefore, experiment can be performed at laboratory temperature so the kinetic manifold does not need to be thermostated during measurement.

**Kinetics of the Reaction**

Under the above-described optimum conditions, the absorbance-time curves for the reaction at varying NAC concentrations \((6.0 \times 10^{-7} \text{ to } 8.0 \times 10^{-5}\) mol L\(^{-1}\)) with a fixed analytical concentrations of \(\text{Cu}^{II}\) \((1.0 \times 10^{-4}\) mol L\(^{-1}\)) and neocuproine \((2.4 \times 10^{-4}\) mol L\(^{-1}\)) were generated (Figure 2).

**Quantitation Methods**

*Initial Rate Method.*

According to experimental data, the initial rates of the proposed reaction with NAC would follow a pseudo-
first order and were found to obey the following equation:

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

where $K$ is the reaction rate, $A$ absorbance, $t$ the measuring time, $k'$ the pseudo-first order apparent rate constant, $c$ the molar concentration of NAC and $n$ the order of the reaction. The logarithmic form of the above equation is written as

$$\log K = \log (\Delta A/\Delta t) = \log k' + n \log c. \tag{3}$$

Mathematically the initial reaction rates ($K$) were determined from the slopes of absorbance-time curves ($\Delta A/\Delta t$). The logarithms of the reaction rates ($\log K$) were plotted as a function of logarithms of the NAC concentrations ($\log c$) (Figure 3).

**Fixed-time Method.**

In this method, the absorbance of the reaction solution containing varying amounts of NAC was measured at a pre-selected fixed time. Calibration plots of the absorbance versus the concentration of NAC were established at fixed periods of time for the reaction. During the optimization of the proposed method, the various time intervals for the absorbance measuring were observed. The best linearity and wider concentration range were achieved at the beginning of the reaction, probably because the reaction product is not stable, especially at higher concentrations. Therefore, the time interval of 1 minute after the analyte addition was chosen.

**Linear Dynamic Range**

Regression analysis, using the method of least squares, was performed to evaluate the slope, intercept and to determine the coefficient of correlation ($r^2$). For the both methods graphs were linear for NAC concentrations ranging from $6.0 \times 10^{-7}$ to $8.0 \times 10^{-5}$ mol L$^{-1}$. For the initial rate method the intercept $\log k' = 2.993$ and coefficient $r^2 = 0.9998$ were calculated. The calculated value of $n$ (slope) 1.018 (≈ 1) in the regression equation (See Figure 3.) confirmed the first-order reaction with respect to the NAC concentration. For the fixed-time method calibration graph obeyed the equation: $y = 7111x - 0.0027$, where $y$ is the absorbance and $x$ is NAC concentration expressed in mol L$^{-1}$. For the both methods the calculated limit of detection (LOD) and the limit of quantification (LOQ) had the same values. LOD was $1.6 \times 10^{-7}$ mol L$^{-1}$ based on 3$\sigma$ of the blank solution ($n = 12$). LOQ, calculated as 10$\sigma$ of the 12 blank solution, was $5.4 \times 10^{-7}$ mol L$^{-1}$.

**Interferences Studies**

The effect of some possible interfering species was studied by analysing synthetic sample solutions containing 40.0 $\mu$M of NAC and various concentrations of interfering substances. The influence of excipients that can commonly accompany NAC in pharmaceutical formulations was also studied. The 500-fold excess (20.0 mM) of examined ions (Na$^+$, K$^+$, NO$_3^-$) and organic acids (boric acid, tartaric acid, citric acid) did not interfere. An error of 5 % was considered to be tolerable. Similarly, 100-fold molar excess of sugars (glucose, fructose, sucrose and lactose) showed no effect on the results. It should be emphasized that this contaminant/analyte concentration ratio studied is much higher than those normally found in commercial pharmaceutical products.

Although redox-active colorimetric reagents generally have limited selectivity – depending on their formal potential and reaction kinetics, the relative selectivity of Cu(II)-neocuproine reagent was verified by its lack of attack on frequently present reductants: citric acid and reducing sugars. Interferences are, of course, expected from the substances possessing strong reduction properties, e.g. hydrazine or ascorbic acid. However, reducing substances are usually not present in pharmaceutical preparations containing NAC as an active substance.

**Accuracy**

The accuracy of the proposed methods was checked by carrying out recovery studies. In this procedure, known amounts of NAC standard were added to preanalysed pharmaceutical formulation before determination by the recommended methods. The recoveries were in the range from 99 to 103 % for the both methods, as shown in Table 1. These results prove good accuracy of the proposed methods and the absence of interferences from common excipients. This indicates that the proposed kinetic methods are reliable for the determination of NAC in pharmaceutical preparations.

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Application

The applicability of the proposed methods for the determination of NAC was checked by analysing several types of pharmaceutical formulations (syrup, dispersible tablets and tablets), containing different amounts of NAC. The concentration of NAC was computed from its corresponding regression equations. The results of the proposed methods (initial rate and fixed time) were compared with those obtained by official method recommended by the European Pharmacopoeia based on titration of NAC with iodine (Table 2).

Performance characteristics of reported spectrophotometric methods, for the determination of NAC using classic, kinetic or flow injection setup and the proposed method, are compared in Table 3.

Among the described spectrophotometric procedures the most sensitive approaches to the determination of NAC are based on the reduction properties of analyte using redox-active colorimetric reagents. There are few developed spectrophotometric methods based on the reduction of the FeIII to FeII with NAC, followed by the complexation of FeII with different reagents: 1,10-phenanthroline (phen), ferrozine, ferrozine, 2,4,6-trypyridyl-s-triazine (TPTZ), hexacyanoferrate(III) and ferricyanide.

The authors have developed kinetic spectrophotometric methods for determination of NAC using FeIII-phen reagent and FeIII-TPTZ reagent. The reported standard potential of CuII/CuI couple in the solution of neocuproine (0.603 V) is comparable to the formal potential for the optimized conditions, of the FeIII/FeII couple in solution with TPTZ (0.578 V). The potentials of the above-mentioned redox-active colorimetric reagents are both lower than a formal potential of FeIII/FeII couple in solution with 1,10-phenanthroline (1.197 V). The Cu(II)-neocuproine reagent is milder and therefore more selective oxidant than FeIII-phenanthroline reagent. Only the reducing substances with standard (formal) potentials lower than 0.6 V would have the thermodynamic predisposition to interfere in the proposed method. On the other hand, the enhanced sensitivity of CuII-neocuproine reagent is probably due to kinetic reasons. The CuII-neocuproine reagent is an immediate product of one-electron reduction of the reagent with NAC, while the described procedure with FeIII-TPTZ based on the reduction of iron(III) to iron(II) by NAC, followed by TPTZ chelation of the formed FeII requires a longer time. The additional advantage of CuII-neocuproine reagent is the absence of interference by PO43– ions. The PO43– ions interfere in methods based on the reduction of the FeIII to FeII because the phosphates make a stable complex with the FeIII and therefore the proposed couple redox-complexation reaction would not proceed. Moreover, the proposed method is proceeding in the acid medium i.e., Britton-Robinson buffer solution (pH = 3.0), where the analyte solution is more stable and the reduction properties of most interferences is reduced, thereby adding some selectivity to the proposed method.

Table 1. Evaluation of the accuracy of the initial rate and fixed time methods of the proposed kinetic spectrophotometric method for determination of NAC

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial rate method</th>
<th></th>
<th></th>
<th>Fixed time method</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added / µg mL⁻¹</td>
<td>Found¹ / µg mL⁻¹</td>
<td>Recovery / %</td>
<td>Added / µg mL⁻¹</td>
<td>Found¹ / µg mL⁻¹</td>
<td>Recovery / %</td>
</tr>
<tr>
<td>Fluimukan Akut Junior Syrup (b)</td>
<td>0.0</td>
<td>100.8 ± 0.2</td>
<td>–</td>
<td>0.0</td>
<td>100.2 ± 0.3</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>151.0 ± 0.5</td>
<td>100.3</td>
<td>50.0</td>
<td>149.7 ± 0.4</td>
<td>98.9</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>203.1 ± 0.8</td>
<td>102.4</td>
<td>100.0</td>
<td>199.1 ± 0.7</td>
<td>98.9</td>
</tr>
<tr>
<td></td>
<td>150.0</td>
<td>248.7 ± 0.9</td>
<td>98.6</td>
<td>150.0</td>
<td>250.1 ± 0.9</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>303.8 ± 1.1</td>
<td>101.5</td>
<td>200.0</td>
<td>305.4 ± 0.9</td>
<td>102.6</td>
</tr>
</tbody>
</table>

¹ Average of three determinations ± SD.
(b) Syrup containing NAC 100 mg in 5 mL and excip.

Table 2. Content of NAC in pharmaceutical formulations determined by the official method and the proposed methods (initial rate and fixed time)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial rate method / Fixed time method / Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluimukan granules (b)</td>
<td>103.4 ± 0.5 / 102.6 ± 0.4 / 101.5 ± 0.8</td>
</tr>
<tr>
<td>Fluimukan Akut Junior Syrup (c)</td>
<td>100.8 ± 0.2 / 100.2 ± 0.3 / 100.3 ± 0.6</td>
</tr>
<tr>
<td>Fluimukan Akut Dispersible Tablets (d)</td>
<td>605.2 ± 0.6 / 604.5 ± 0.9 / 605.6 ± 0.9</td>
</tr>
</tbody>
</table>

¹ Average of three determinations ± SD.
(b) Granules containing NAC 100 mg and excip.
(c) Syrup containing NAC 100 mg in 5 mL and excip.
(d) Dispersible tablets containing NAC 600 mg and excip.
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Reagent(s) used</th>
<th>$\lambda_{\text{max}}$ / nm</th>
<th>Beer's law range / μM</th>
<th>LOD / μM</th>
<th>Measuring mode:</th>
<th>RSD / %</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>iron (III) and 1,10-phenanthroline</td>
<td>515</td>
<td>4.5 – 80</td>
<td>no data</td>
<td>classic (20 min)</td>
<td>0.34</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>6</td>
<td>α-phthalaldehyde and isoleucine</td>
<td>335</td>
<td>500 – 49000</td>
<td>no data</td>
<td>classic</td>
<td>1.50</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>32</td>
<td>palladium(II) chloride</td>
<td>375</td>
<td>4000 – 653000</td>
<td>1630</td>
<td>classic (10 min)</td>
<td>1.92</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>25</td>
<td>cobalt (III)-ethylenediaminetetraacetate complex, hypophosphite, palladium(II) chloride</td>
<td>540</td>
<td>0.16 – 16.7</td>
<td>no data</td>
<td>kinetic</td>
<td>2.10 – 3.30</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>33</td>
<td>palladium(II) chloride</td>
<td>380</td>
<td>50 – 5000</td>
<td>10</td>
<td>flow injection (45 h$^{-1}$)</td>
<td>1.40</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>7</td>
<td>enzyme acylase, ninhydrin</td>
<td>550</td>
<td>no data</td>
<td>no data</td>
<td>classic</td>
<td>no data</td>
<td>Tissue</td>
</tr>
<tr>
<td>8</td>
<td>iron (III) and ferrozine</td>
<td>562</td>
<td>20 – 6000</td>
<td>no data</td>
<td>classic (10 min)</td>
<td>1.53</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>28</td>
<td>iron (III) and 1,10-phenanthroline</td>
<td>510</td>
<td>3.5 – 430</td>
<td>0.67</td>
<td>flow injection (60 h$^{-1}$)</td>
<td>1.50</td>
<td>Pharmaceuticals</td>
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<tr>
<td>34</td>
<td>bromine</td>
<td>400</td>
<td>160 – 1600</td>
<td>80</td>
<td>flow injection (60 h$^{-1}$)</td>
<td>1.20</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>9</td>
<td>potassium iodate and leucoxylenecyanol</td>
<td>613</td>
<td>200 – 1600</td>
<td>200</td>
<td>classic</td>
<td>10.0</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>23</td>
<td>iron (III) and 1,10-phenanthroline</td>
<td>510</td>
<td>8 – 80</td>
<td>2.06</td>
<td>kinetic</td>
<td>no data</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>26</td>
<td>4-chloro-7-nitrobenzo-2-oxa 1,3 – diazole</td>
<td>424</td>
<td>200 – 220000</td>
<td>no data</td>
<td>kinetic</td>
<td>no data</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>24</td>
<td>iron (III) and 2,4,6-trypyridyl-s-triazine</td>
<td>593</td>
<td>1 – 100</td>
<td>0.17</td>
<td>kinetic</td>
<td>no data</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>35</td>
<td>iron (III) and 2,4,6-trypyridyl-s-triazine</td>
<td>593</td>
<td>4 – 100</td>
<td>1.00</td>
<td>kinetic</td>
<td>no data</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>29</td>
<td>iron (III) and hexacyanoferrate(III)</td>
<td>700</td>
<td>no data</td>
<td>10</td>
<td>flow injection (70 h$^{-1}$)</td>
<td>no data</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>30</td>
<td>iron (III) and ferricyanide</td>
<td>735</td>
<td>1 – 100</td>
<td>0.3</td>
<td>flow injection (60 h$^{-1}$)</td>
<td>2.50</td>
<td>Pharmaceuticals</td>
</tr>
<tr>
<td>36</td>
<td>ferroin and cerium(IV)</td>
<td>500</td>
<td>6.5 – 130</td>
<td>5.0</td>
<td>flow injection (60 h$^{-1}$)</td>
<td>1.40</td>
<td>Pharmaceuticals</td>
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<tr>
<td>37</td>
<td>α-phthalaldehyde and isoleucine</td>
<td>335</td>
<td>100 – 1000</td>
<td>no data</td>
<td>flow injection (126 h$^{-1}$)</td>
<td>0.60</td>
<td>Pharmaceuticals</td>
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<tr>
<td>present work</td>
<td>copper (II)-neocuproine</td>
<td>458</td>
<td>0.6 – 80</td>
<td>0.16</td>
<td>kinetic</td>
<td>0.50</td>
<td>Pharmaceuticals</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the spectrophotometric methods for NAC determination.
CONCLUSION

A simple, rapid and sensitive kinetic spectrophotometric method for the determination of NAC has been successfully developed and validated. This method is based on the redox reaction where the analyte reduces Cu^{II}-neocuproine complex to Cu'-neocuproine complex. The proposed reaction is fast, temperature independent and can proceed in a broad pH range. Also, the kinetic of the proposed reaction is promising for the development of the flow injection method, what is the further aim of our research. The initial rate and fixed time (at 1 min) methods were utilized in this experiment. The proposed methods have several advantages over the previously reported kinetic spectrophotometric methods: wide linearity range of the calibration curve (over two decades, 0.6–80 μM), higher sensitivity, selectivity, speed (1 min for the proposed fixed time method) and straightforwardness compared to Ref. 25. In addition, proposed methods are sensitive enough to enable determination of near nanomole amounts of the NAC without expensive instruments. Application of the proposed methods to the determination of NAC in pharmaceutical samples has produced excellent results in terms of accuracy, as the recoveries were in the range from 99 to 103%.

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