ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2023**, *96*(2), 91–97
Published online: October 29, 2023
DOI: 10.5562/cca4016

HKD 1926

Spectrophotometric Determination of *N*-acetyl-L-Cysteine in Pharmaceutical Formulations by Flow Injection and Sequential Injection Analysis: Comparison of the Methods

Maja Biocic,[1,*] Deni Karabatić,[2] Dea Tomić,[2] Lea Kukoc-Modun[1]

[1] University of Split, Faculty of Chemistry and Technology, Department of Analytical Chemistry, Ruđera Boškovića 35, 21000 Split, Croatia

[2] University of Split, School of Medicine, Department of Pharmacy, Šoltanska 2, 21000 Split, Croatia

* Corresponding author's e-mail address: maja.biocic@ktf-split.hr

RECEIVED: July 13, 2023 * REVISED: October 12, 2023 * ACCEPTED: October 16, 2023

Abstract: *N*-acetyl-L-cysteine (NAC), a sulfhydryl-containing compound, is mainly used as a mucolytic and as an antidote for acetaminophen overdose. Flow injection and sequential injection systems were designed and optimized with the aim of providing precise, accurate and reliable flow methods for NAC determination in pharmaceuticals with very low sample and reagent consumption. Proposed methods are based on a redox reaction wherein NAC reduces a complex of Cu(II) and bathocuproine disulfonate (BCS) to orange $[Cu(BCS)_2]^{3-}$ complex, which absorption was measured at λ_{max} = 483 nm. The optimized FIA and SIA configuration yielded a linear calibration curve with correlation coefficients (R^2 = 0.9999 and R^2 = 0.9996) in the concentration range of $3.0 \times 10^{-7} - 3.0 \times 10^{-5}$ mol L^{-1} and analytical frequency of 120 h^{-1} for the FIA method and $4.0 \times 10^{-7} - 4.0 \times 10^{-5}$ mol L^{-1}, at sampling rate 60 h^{-1} for the SIA method. The proposed flow methods were successfully applied for the determination of NAC in pharmaceutical products, as the results showed good agreement with the standard method prescribed by Pharmacopoeia. Recoveries were in the range from 98.4 % to 101.9 % for the FIA method and from 97.2 % to 101.8 % for the SIA method.

Keywords: *N*-acetyl-L-cysteine, flow injection analysis, sequential injection analysis, spectrophotometric determination, pharmaceuticals.

INTRODUCTION

N-ACETYL-L-CYSTEINE (NAC) is an acetylated form of the amino acid L-cysteine. It is a very important antioxidant thiol compound with a small molecular weight. NAC is employed as a mucolytic agent commonly used to reduce sputum viscosity in chronic asthma and bronchitis and reduce the viscosity of ophthalmic secretions.[1] It was originally patented in 1960, and its use in medicine was first reported in 1967.[2] Clinically it has been used in cystic fibrosis since 1969.[3] Since then, NAC use has been expanded to acetaminophen overdose and lung disease, and its role has been expanding.[4] Due to its pharmaceutical and clinical importance, there is a need to develop fast and sensitive methods for the determination of NAC, particularly in drug analysis. British Pharmacopeia recommends iodometric titration for the determination of NAC in pharmaceuticals as a standard method.[5]

Analytical quality is a key factor in the success of programs for development, production and quality control of pharmaceuticals. In recent years the regulations related to the quality control of medicines required by international pharmacopoeias have become strict, demanding modern pharmaceutical analysis to produce rapid, reliable and economical results.[6] Since the first paper on flow injection analysis (FIA) appeared in 1975,[7] FIA has proven to be a powerful instrumental analytical technique for drug analysis. The advantages of this technique are mainly its simplicity, cost performance, high through capacities and flexibility.[8] Sequential injection analysis (SIA), the second-generation flow-based technique, shares similarities with FIA. Retaining the benefits of the FIA, the methodology of SIA enables full computerization of the execution of analysis.[9] Moreover, the reduction of sample and reagent consumption is a significant SIA advantage, which reduces costs, especially when dealing with high-cost chemicals or hazardous reagents.

Flow analysis methods for the determination of NAC in pharmaceuticals, using different detectors, are listed and compared in Table 1.

From the analytical parameter data listed, it is apparent that both the FIA and SIA spectrophotometric methods enable the analysis of a significantly larger number of samples with a broader concentration range for the determination of NAC.

The methods listed in Table 1 are based on redox reactions and/or combined redox and complexometric reactions. There is a need for new flow methods that can reduce the consumption of analytes and reagents and thus reduce the production of waste while keeping the sampling rate high.

The main objective of the present work is to develop and validate new flow methods for the determination of NAC in pharmaceutical formulations that meet laboratory quality control requirements. Based on a one-step colorimetric redox reaction using Cu(II) and bathocuproine disulfonate (BCS) reagents in Britton Robinson buffer at pH 3.0,[21] both the FIA and SIA methods are characterised with respect to their simplicity, versatility, low sample and reagent consumption and robustness.

EXPERIMENTAL

Reagents and Solutions

During this experimental work Milli-Q (Millipore) double deionized water was used. All chemicals were of analytical reagent grade.

The stock solution of 0.01 mol L^{-1} *N*-acetyl-L-cysteine (NAC) was prepared by dissolving 0.0816 g of NAC (Acros Organics, New Jersey, USA) into Britton-Robinson buffer solution (pH = 2.0) and diluted to 50.0 mL volume. The prepared stock solution was stable for at least 30 days stored at 4 °C in dark bottle. Working standards of lower concentration were prepared daily, by diluting the above-mentioned stock solutions with Britton-Robinson buffer solution (pH = 3.0).

A stock solution of 0.001 mol L^{-1} Cu(II) was prepared by dissolving 0.0125 g of copper sulphate pentahydrate (Kemika, Zagreb, Croatia) in deionized water and diluting to the mark in a 50.0 mL standard flask.

0.002 mol L^{-1} bathocuproine disulfonate solution (BCS) was prepared by dissolving 0.0564 g of its disodium salt (Sigma – Aldrich, Germany) up to 50.0 mL volume with deionized water.

The Britton-Robinson buffer solution (0.04 mol L^{-1}, pH = 2.0) was prepared by dissolving 4.95 g of boric acid, mixing with 4.80 g of glacial acetic acid and 5.46 g of phosphoric acid and diluting with deionized water up to 2.0 L. pH values were adjusted using 2.0 mol L^{-1} sodium hydroxide solution. pH adjustments and measurements were carried out with a Mettler Toledo SevenMulti potentiometer (Mettler Toledo, Schwerzenbach, Switzerland) equipped with a combined glass electrode Mettler Toledo InLab®413.

Commercially available pharmaceutical products in the form of granules and tablets were selected and analyzed in this work: Fluimukan Junior 100 mg of NAC (Sandoz d.o.o., Zagreb, Hrvatska), Fluimukan 200 mg of NAC (Sandoz d.o.o., Zagreb, Hrvatska) and Naxil Forte 600 mg of NAC (Belupo d.d., Koprivnica, Hrvatska). Three tablets of solid preparations were weighed, thoroughly ground into powder and mixed. Granules and tablets were dissolved and diluted by deionized water to the nominal volume in a

Table 1. The comparison of the analytical parameters of the previously developed flow methods using different detectors for the determination of NAC in pharmaceuticals.

Reference	Reagents	Analytical method (Sampling rate)	Beer's Law / mol L^{-1}	LOD / mol L^{-1}	RSD / %	Detector
[10]	Iron(III) and 1,10-phenantroline	FIA (60 h^{-1})	3.5×10^{-6} – 4.3×10^{-4}	6.3×10^{-7}	1.50	Spectrophotometric
[11]	Bromine	FIA (60 h^{-1})	1.6×10^{-4} – 1.6×10^{-3}	8.0×10^{-5}	1.20	Spectrophotometric
[12]	Iron(III) and 2,4,6-tripyridyl-*s*-triazine (TPTZ)	FIA (60 h^{-1})	6.0×10^{-6} – 2.0×10^{-4}	2.0×10^{-6}	0.29	Spectrophotometric
[13]	Iron(III) and hexacyanoferrate(III)	FIA (70 h^{-1})	3.0×10^{-5} – 2.0×10^{-4}	1.0×10^{-5}	No Data	Spectrophotometric
[14]	Iron(III) and ferricyanide	FIA (60 h^{-1})	1.0×10^{-6} – 1.0×10^{-4}	3.0×10^{-7}	2.50	Spectrophotometric
[15]	Cerium(IV) and ferroin	FIA (60 h^{-1})	6.5×10^{-6} – 1.3×10^{-4}	5.0×10^{-6}	1.40	Spectrophotometric
[16]	zink(II) phosphate	FIA (60 h^{-1})	3.0×10^{-5} – 1.5×10^{-4}	8.0×10^{-6}	0.50	Spectrophotometric
[17]	copper(II) and neocuproine	FIA (180 h^{-1})	6.0×10^{-7} – 4.0×10^{-5}	1.4×10^{-7}	0.50	Spectrophotometric
[18]	silver nitrate	FIA (60 h^{-1})	1.0×10^{-4} – 1.0×10^{-3}	5.0×10^{-5}	2.00	Turbidimetric
[19]	boron-doped diamond electrode	FIA (No Data)	5.0×10^{-7} – 5.0×10^{-5}	1.0×10^{-8}	4.10	Amperometry
[20]	monobromobimane	SIA (36 h^{-1})	1.5×10^{-4} – 4.6×10^{-4}	1.3×10^{-6}	1.50	Fluorimetry

FIA - Flow Injection Analysis; SIA - Sequential Injection Analysis; LOD - Limit of Detection; RSD - Relative Standard Deviation.

500 mL volumetric flask. The solutions prepared in this way were analyzed within 24 hours and in the given time period were found to be stable.

Iodine solutions were prepared and standardized according to the literature for the validation part of the experiment.[5]

Apparatus and Procedure

For both FIA and SIA signal detection, a double beam UV-Vis spectrophotometer (Shimadzu UV – 1601, Kyoto, Japan) equipped with 10 mm optical path flow cell (Hellma, Jamaica, NY) with inner volume of 160 and 80 µL, respectively, connected to a personal computer equipped with Hyper UV-Vis software, Shimadzu, was used. The fluid transport through the system took place through flexible PTFE tubing. The absorbance of the reduced orange $[Cu(BCS)_2]^{3-}$complex was measured at the maximum absorption wavelength of 483 nm.

The FIA instrumentation was equipped with a peristaltic pump (Ismatec, Zurich, Switzerland) and a 6-port rotary injection valve (Rheodyne, Model 5020, Anachem, Luton, UK) connected to a confluence point and reaction coil (Figure 1 (a)). A more detailed description of the FIA manifold was reported previously.[17,21]

SIA instrumentation consisted of a bi-directional syringe free pump Cheminert® M50 (VICI Valco Instruments, Houston, Texas, USA) and a multi position (10-port) selection valve (VICI Valco Instruments, Houston, Texas, USA) connected to a holding coil (0.5 mm inner diameter) and reaction coil (0.8 mm inner diameter). The SIA system used for the determination of NAC is shown in Figure 1 (b).

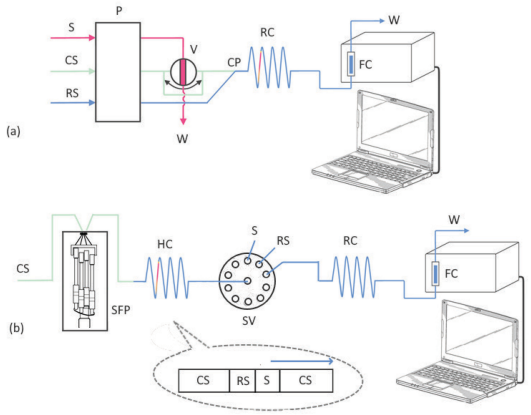

Figure 1. Schematic diagram of the FIA system (a) and SIA system (b) for NAC determination: Sample solution (S); carrier stream (CS), reagent stream (RS), peristaltic pump (P); injection valve (V); syringe free pump (SFP), holding coil (HC), selection valve (SV), reaction coil (RC), flow cell (FC), waste (W); Inset: Sequence of the solutions in the holding coil and the direction of the flow in the SIA system.

The central inlet of the 10-port valve was connected to a holding coil with an inner diameter of 0.5 mm and an inner volume of 500 µL. The high precision syringe free pump Cheminert®M50 was used to aspirate the samples and reagents. A computer was utilized to control the operation of the components of the SIA system and the commands were entered into the program according to the following procedure:

1. In this step, 3000 µL of deionized distilled water were delivered at a 6000 µL min^{-1} flow rate through port 8 of the selection valve. This process was employed to adjust the blank signal of the detector.
2. To load the holding coil, 200 µL of reagent and 250 µL of standard or sample solution were passed through ports 7 and 6, respectively.
3. After reaction of NAC with the $[Cu(BCS)_2]^{2-}$ reagent, deionized water was used to flush the content of the holding coil at a flow rate of 6000 µL min^{-1} through port 8 to the reaction coil, thus delivering the reaction zone to the detector for absorbance reading and subsequent re-establishment of the baseline.

Steps 1–3 of the procedure were repeated three times (triplicate measurement).

RESULTS AND DISCUSSION

The development of the orange colour in the visible part of the spectrum, is based on a one step redox reaction in which NAC reduces $[Cu(BCS)_2]^{2-}$ complex to $[Cu(BCS)_2]^{3-}$ complex and the absorbance is measured at 483 nm.[21]

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

During the development of the method, the chemical parameters and the parameters of the flow system were optimized using a univariate method.

Optimization of the Chemical Parameters

Preliminary FIA and SIA studies included optimization of chemical parameters: pH value and reagent molar ratio.

The effect of pH value was studied in both flow systems in the range of 2.0 to 8.0 using the Britton Robinson buffer (acetate-borate-phosphate buffer). The maximum signal was reached at pH 3.0. No significant increase in signal was observed at higher pH values. Since NAC is a thiol compound, it is more stable in an acidic medium, and thus it showed the highest stability at lower pH values. Therefore, a pH value of 3.0 was chosen as the optimal value for further measurements.

The optimization of the molar ratio of BCS and Cu^{2+} was performed in such a way that the concentration of Cu^{2+} was kept constant and the concentration of BCS was

changed. The molar ratio of BCS and Cu^{2+} varied in the range of 1 : 1 to 3 : 1. The results in the FIA system showed that the signal changed slightly at higher ratios, so 1 : 1 was selected as the optimal ratio. In the SIA system, the results showed that as the molar ratio increased, the absorbance also increased and reached a maximum when the molar ratio of BCS and Cu^{2+} was 2 : 1.

Based on previous kinetic measurements, the proposed redox reaction was found to be temperature independent. Therefore, for practical reasons (no need for thermostating the flow system), a room temperature of 25 °C was chosen for this method.

Optimization of the FIA and SIA Systems Parameters

OPTIMIZATION OF THE FIA SYSTEM PARAMETERS

The carrier flow rate determines the frequency of measurements and controls the dispersion and chemical kinetics, which affect the sensitivity and sampling rate. The effect of the carrier flow rate in the FIA system on signal height was investigated in the range of 1 to 6 mL min^{-1} using a peristaltic pump. 6 mL min^{-1} was chosen as the optimal value due to signal stability and satisfactory return to baseline.

The effect of the flow rate of the reagent solution on signal height in the FIA system was examined in the range of 2.0 to 6.0 mL min^{-1} using pump tubes of different internal diameters. The highest and stable signal was obtained with a reagent flow rate of 2.0 mL min^{-1} which was selected as the optimal value considering low reagent consumption.

Optimization of the injected sample volume in the FIA system was performed using sample injection valve loops with different volumes (from 100 to 1000 µL). As the injection volume increased, the signal height increased up to 500 µL and thus the sensitivity of the method increased. Consequently, the width of the peaks, and baseline also increased, which affects the frequency of measurement since it took more time for the signal to return to the baseline. Therefore, 500 µL was chosen as the optimal volume as a compromise between the sensitivity of the method and the frequency of measurement.

The effect of the reaction coil length was studied in the range of 30 to 400 cm for the FIA system. In order not to compromise on the completeness of the chemical reaction, the reaction coil length of 50 cm for the FIA system was selected.

OPTIMIZATION OF THE SIA SYSTEM PARAMETERS

Optimization of the aspiration sequence in the SIA system was examined using the aspiration order of sample and reagent solutions into the holding coil. Two measurements were performed: In the first one, the reagent solution was aspirated into the holding coil, followed by the sample solution; in the other, the aspiration order was reversed. The sequence in which the reagent solution was aspirated first and then the sample solution showed the highest peak absorbance and the best precision. This order of injection was maintained for further measurements.

Flow rates ranging from 1 mL min^{-1} to 8 mL min^{-1} were investigated using the syringe free pump in the SIA system. The flow rate at 6.0 mL min^{-1} showed maximum signal height with better repeatability. This proved a nearly insignificant standard deviation (9.24×10^{-4}) compared with the standard deviation of the signal at flow rate 2.0 mL min^{-1} (4.78×10^{-3}) of nearly similar height. The time between two injections of sample was at least two times shorter than (120 s in triplicate) in comparison to the flow rate of 2.0 mL min^{-1} (300 s in triplicate). A 6 mL min^{-1} flow rate of the carrier solution was selected, as a compromise between return time (*T*′) and reagent consumption, sampling frequency, and sensitivity. At this flow rate, maximum peak absorbance was recorded with an acceptable peak width and time to return to baseline.

The aspirated volumes of reagent and sample used for the optimization of the SIA system ranged from 50 µL to 450 µL. After measurement, the optimal reagent volume was set at 200 µL. Further increase of volume did not increase the analytical signal but it increased the amount of waste. Increasing the aspiration of sample volume up to 250 µL, the analytical signal increased and higher volumes had an insignificant effect. Higher volumes also decreased the sampling frequency and required a longer runtime so the sample volume of 250 µL was chosen for subsequent studies as a compromise between the sensitivity of the method and the sampling frequency.

Since the reaction in the SIA system starts in the holding coil, two volumes of holding coils were tested (500 µL and 1000 µL). The 500 µL volume was selected as optimal hence there was no difference in signal height, and this volume of the holding coil was sufficient for the total aspirated volume of sample and reagent. In addition, the carrier consumption was lower with better signal repeatability.

The effect of the reaction coil length was studied in the range of 30 to 120 cm for the SIA system. The signal with the highest peak absorbance and the best repeatability was selected. Thus, the reaction coil length of 30 cm was chosen as the optimal value.

Analytical Characteristics of the Proposed Flow Methods for the Determination of NAC

Considering the previously optimized experimental parameters (Table 2), the flow methods were used to construct calibration curves by determining NAC standards in the range of 3.0×10^{-7} and 1.0×10^{-4} mol L^{-1}.

Linearity was achieved in the concentration range between 3.0×10^{-7} and 3.0×10^{-5} mol L^{-1} for the FIA method

(R^2 = 0.9999) and between 4.0 × 10^{-7} and 4.0 × 10^{-5} mol L^{-1} for the SIA method (R^2 = 0.9996, Fig. 2).

Limit of detection (LOD) and limit of quantification (LOQ) were calculated (Table 3) according to the following expressions: LOD = 3 σ / s and LOQ = 10 σ / s, where σ is the standard deviation of ten reagent blank signals and s is the slope of the calibration curve.

The relative standard deviation (RSD) for ten replicate determination of NAC was 0.61 % at 3.0 × 10^{-5} mol L^{-1} by the FIA method. The SIA method also showed high repeatability with an acceptable values of RSD, that was, 2.62 % at 4.0 × 10^{-5} mol L^{-1}.

Table 2. Optimization of the FIA and SIA parameters for the determination of NAC.

Parameters studied	Studied range	Optimal conditions	
		FIA	SIA
Temperature / °C	20; 25; 30; 40; 50	25	25
pH	2.0; 3.0; 4.0; 5.0; 6.0; 7.0; 8.0	3.0	3.0
n(BCS) : n(Cu^{2+})	1:1; 2:1; 3:1	1:1	2:1
Aspiration sequence	RS, S; S, RS	–	RS, S
Carrier flow rate / µL min^{-1}	1000; 2000; 3000; 4000; 5000; 6000; 7000; 8000	6000	6000
Reagent flow rate / µL min^{-1}	2000; 4000; 6000	2000	–
Sample injection volume / µL	100; 200; 250; 500; 1000	500	–
Aspiration of reagent volume /µL	50; 100; 150; 200; 250; 300; 350; 400; 450	–	200
Aspiration of sample volume /µL	50; 100; 150; 200; 250; 300; 350; 400; 450	–	250
Volume of holding coil / µL	500; 1000	–	500
Length of the reaction coil / cm	30; 40; 50; 60; 70; 100; 120; 400	50	30

RS – Reagent Solution; S – Sample Solution.

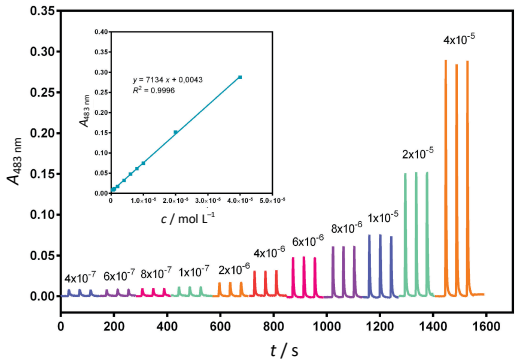

Figure 2. Siagram and calibration curve for the spectrophotometric determination of NAC in the concentration range of 4.0 × 10^{-7} mol L^{-1} to 4.0 × 10^{-5} mol L^{-1}. Experimental conditions: c(Cu^{2+}) = 6.4 × 10^{-4} mol L^{-1}; c(BCS) = 3.2 × 10^{-4} mol L^{-1}; n(BCS) : n(Cu^{2+}) = 2 : 1; t = 25 °C; pH = 5.0; aspiration sequence = RS, S; carrier flow rate = 6000 µL min^{-1}; reagent injection volume = 200 µL; analyte injection volume = 250 µL; volume of holding coil = 500 µL; length of the reaction coil = 30 cm.

Effect of Ions/Interferences

The influence of interfering substances on the determination of NAC was investigated using the proposed methods under optimized conditions (Table 2). To examine the selectivity of the FIA and SIA methods, synthetic NAC samples (3.0 × 10^{-5} mol L^{-1} and 4.0 × 10^{-5} mol L^{-1}) containing substances commonly used in pharmaceutical formulations were analyzed. The tolerance limit was defined as the molar ratio of NAC to the interfering species that had an error of less than ± 5 %.

Table 3. Analytical characteristics of the developed FIA and SIA methods for the determination of NAC.

Parameters studied	FIA method	SIA method
Linear range / mol L^{-1}	3.0 × 10^{-7} – 3.0 × 10^{-5}	4.0 × 10^{-7} – 4.0 × 10^{-5}
Regression equation	y = 8660x – 0.0028	y = 7134x + 0.0043
R^2	0.9999	0.9996
LOD / mol L^{-1}	9.0 × 10^{-8}	1.2 × 10^{-7}
LOQ / mol L^{-1}	3.0 × 10^{-7}	4.0 × 10^{-7}
RSD / %	0.61	2.62
Sampling frequency / h^{-1}	120	60

Table 4. The effect of possible interfering substances commonly used in commercial pharmaceutical formulations.

Substance	Tolerable molar ratio of NAC to interfering substance where the error is lower than 5 %	
	FIA	SIA
Glucose	1 : 500	1 : 500
Fructose	1 : 500	1 : 250
KNO_3	1 : 500	1 : 500
Lactose	1 : 500	1 : 400
Sucrose	1 : 500	1 : 500
Citric acid	1 : 100	1 : 5
Tartaric acid	1 : 100	1 : 5
Na_2SO_4	1 : 500	1 : 50
Na-citrate	1 : 500	1 : 500
Acetylsalicylic acid	1 : 1	1 : 1

The interference of these substances at concentrations up to 500 times higher than the concentration of the analyte, except for acetylsalicylic acid, which was prepared in the same concentration as the analyte was tested. It should be noted, that the concentration of each one of these substances at which interference occurred was much higher than its concentrations in commercial pharmaceutical preparations.

Accuracy and Analytical Application

The proposed FIA and SIA systems were applied for the determination of spiked NAC concentrations in different pharmaceutical products. Recoveries were in the range from 98.4 % to 101.9 % for the FIA method and 97.2 % to 101.8 % for the SIA method (Table 5).

The results indicated acceptable accuracy of the flow methods and confirmed that the developed flow methods were reliable analytical tools for the determination of NAC in pharmaceutical preparations.

Finally, the optimized flow methods were used for the determination of NAC in the pharmaceutical formulations and the results were compared with those of the standard method according to the Pharmacopoeia.[5] The results depicted in Table 6 showed a very good agreement between the results obtained by the flow and sequential injection methods with a spectrophotometric detector and the results of the iodimetric titration method prescribed by the Pharmacopoeia.

Table 5. Evaluation of the accuracy of newly developed flow methods for the determination of NAC.

Sample	Added / μg mL^{-1}	FIA		SIA	
		Found[(b)] / μg mL^{-1}	Recovery[(b)] / %	Found[(b)] / μg mL^{-1}	Recovery[(b)] / %
Fluimukan Junior granules 100 mg[(a)]	0	101.1 ± 0.6	–	100.3 ± 0.5	–
	50	150.3 ± 0.7	98.4	148.9 ± 0.8	97.2
	100	202.8 ± 0.9	101.7	199.0 ± 1.2	98.7
	150	253.4 ± 1.7	101.5	252.7 ± 1.6	101.6
	200	304.9 ± 2.0	101.9	303.8 ± 2.2	101.8

[(a)] Granules containing NAC 100 mg and excip.
[(b)] Average ± standard deviation (SD) of three determinations per sample.

Table 6. Determination of the mass of NAC in pharmaceutical formulations by the standard method and the newly developed FIA and SIA methods.

Sample	Standard method[(d)] / mg	Proposed method[(d)] / mg	
		FIA	SIA
Fluimukan Junior granules[(a)]	101.3 ± 0.8	100.9 ± 0.6	101.5 ± 1.0
Fluimukan Dispersible Tablets[(b)]	201.9 ± 1.2	201.5 ± 1.1	200.9 ± 1.4
Naxil Forte Dispersible Tablets[(c)]	602.0 ± 1.7	603.2 ± 1.8	603.4 ± 2.1

[(a)] Granules containing NAC 100 mg and excip.
[(b)] Dispersible tablets containing NAC 200 mg and excip.
[(c)] Dispersible tablets containing NAC 600 mg and excip.
[(d)] Average ± standard deviation (SD) of three determinations per sample.

Comparison Between the Newly Developed FIA and SIA Methods for the Determination of NAC

The two present methods, FIA and SIA, allowed rapid, simple and inexpensive analytical determination of NAC in pharmaceutical products. Since they utilize a spectrophotometric detector, they are accessible to most laboratories.

An analytical comparison between the developed FIA and SIA methods for the determination of NAC (Table 3) showed that the sensitivity and reproducibility of analysis were higher for the FIA method (LOD = 9 × 10^{-8} mol L^{-1}; RSD = 0.61 %), as was the sampling rate (120 h^{-1}). For the SIA method, there was a reduction in the sampling rate (60 h^{-1}) because the time required for the analysis of a given sample was not only the time needed for the reaction and the measurement to take place but also the time spent for the aspiration of the sample and reagent solutions. This is the main SIA limitation related to the FIA technique. On the other hand, the application of the SIA method reduced sample consumption twice (Table 2).

Another advantage of the automated SIA method for the determination of NAC presented here is that the reagent consumption was 600 μL per sample, analyzed in triplicate, as opposed to 6000 μL of reagent for the FIA method. Thus, the developed FIA analysis and classical iodimetric titration[5] consume 10 and 100 times more reagents, respectively, compared to the SIA method.

CONCLUSIONS

The newly developed and validated flow methods, FIA (semiautomated) and SIA (automated), for the determination of *N*-acetyl-L-cysteine (NAC) are sensitive, accurate, precise and robust, without interferences from a significant number of excipients accompanying commercial pharmaceutical products and without the need for sample pretreatment. In these methods NAC reduces the $[Cu(BCS)_2]^{2-}$ complex to the orange $[Cu(BCS)_2]^{3-}$ complex in one step reaction. When comparing the flow methods, a time-saving advantage is on the side of the FIA method with a sampling rate twice as high as that of the SIA method. The low sample and reagent consumption, as well as low waste production in the SIA system, led to a reduction in the cost of analysis. In addition, the SIA method was fully automated

and required little operator intervention. The ease of use is particularly justified in developing an automated (SIA) methodology for determining NAC in pharmaceutical formulations. This proves that the advantages of the SIA method outweigh its disadvantages.

The reported flow methods of analysis have been successfully applied for the determination of NAC in pharmaceuticals, with results comparable to those obtained by the standard iodimetric method prescribed in the Pharmacopoeia. Enabling the analysis of a large number of samples with small sample and reagent consumption, both FIA and SIA methods for the determination of NAC are environmentally friendly analytical tools that can be used in quality control laboratories.

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

REFERENCES

[1] G. S. Kelly, *Altern. Med. Rev.* **1998**, *3*, 114–124. https://doi.org/10.1007/978-3-642-72132-8_15

[2] C. B. Lillibridge, J. M. Docter, S. Eidelman, *The Journal of Pediatrics.* **1967**, *71*, 887–889. https://doi.org/10.1016/S0022-3476(67)80019-2

[3] M. Gracey, V. Burke, C. M. Anderson, *Archives of Disease in Childhood*. **1969**, *44*, 404–405. https://doi.org/10.1136/adc.44.235.404

[4] G. K. Schwalfenberg, *J. Nutr. Metab.* **2021**, 9949453. https://doi.org/10.1155/2021/9949453

[5] British Pharmacopoeia. Volume I & II., **2009**, p. 109.

[6] A. M. Pimenta, M. C. B. S. M. Montenegro, A. N. Araujo, J. M. Calatayud, *J. Pharm. Biomed. Anal.* **2006**, *40*, 16–34. https://doi.org/10.1016/j.jpba.2005.10.006

[7] J. Ŕužička, E. H. Hansen, *Anal. Chim. Acta.* **1975**, *79*, 145–157. https://doi.org/10.1016/S0003-2670(01)84761-9

[8] M. I. Evgen'ev, S.Y. Garmonov, L. S. Shakirova, *J. Anal. Chem.* **2001**, *56*, 313–323. https://doi.org/10.1023/A:1016687826266

[9] J. Ŕužička, G. D. Marshall, *Anal. Chem. Acta*. **1990**, *237*, 329–343. https://doi.org/10.1016/S0003-2670(00)83937-9

[10] A. L. T. Fornazari, W. T. Suarez, H. J. Vieira, O. Fatibello-Filho, *Acta Chim. Slov.* **2005**, *52*, 164–167.

[11] W. T. Suarez, H. J. Vieira, O. Fatibello-Filho, *J. Pharm. Biomed. Anal.* **2005**, *37*, 771–775. https://doi.org/10.1016/j.jpba.2004.11.032

[12] L. Kukoc-Modun, I. Plazibat, N. Radić, *Croat. Chem. Acta.* **2011**, *84*, 81–86. https://doi.org/10.5562/cca1753

[13] W. T. Suarez, O. D. Pessoa-Neto, B. C. Janegitz, H. J. Vieira, R. C. Faria, O. Fatibello-Filho, *Anal. Lett.* **2011**, *44*, 2394–2405. https://doi.org/10.1080/00032719.2010.551696

[14] A. Waseem, M. Yaqoob, A. Nabi, *Chem. Res. Chinese U.* **2010**, *26*, 893–898. https://doi.org/10.2116/analsci.26.355

[15] H. J. Vieira, O. Fatibello-Filho, *Quim. Nova.* **2005**, 28, 797–800. https://doi.org/10.1590/S0100-40422005000500012

[16] W. T. Suarez, A. A. Madi, F. C. Vicentini, O. Fatibello-Filho, *Anal. Lett.* **2007**, *40*, 3417–3429. https://doi.org/10.1080/00032710701689040

[17] L. Kukoc-Modun, D. Tsikas, M. Biocic, Nj. Radić, *Anal. Lett.* **2016**, *49*, 607–617. https://doi.org/10.1080/00032719.2014.996811

[18] W. T. Suarez, H. J. Vieira, O. Fatibello-Filho, *J. Braz. Chem. Soc.* **2007**, 18, 1028–1033. https://doi.org/10.1590/S0103-50532007000500023

[19] N. Nantaphol, O. Chailapakul, W. Siangproh, *Electroanalysis* **2014**, *26*, 1024–1030. https://doi.org/10.1002/elan.201400065

[20] P. D. Tzanavaras, T. D. Karakosta, *J. Pharm. Biomed. Anal.* **2011**, *54*, 882–885. https://doi.org/10.1016/j.jpba.2010.11.006

[21] L. Kukoc-Modun, T. Kraljević, D. Tsikas, N. Radić, D. Modun, *Molecules.* **2021**, *26*, 6826–6834. https://doi.org/10.3390/molecules26226826