Original Research

O. Yu. Maslov, S. V. Kolisnyk, M. A. Komisarenko, E. Yu. Akhmedov,
A. O. Koval, T. A. Kostina, O. V. Kolisnyk

National University of Pharmacy of the Ministry of Health of Ukraine,
53 Pushkinska str., 61002 Kharkiv, Ukraine

Metrological Characteristics of the Potentiometric Assay Developed for Determining the Antioxidant Activity of Ascorbic Acid

Abstract

The potentiometric assay for determining the antioxidant activity of ascorbic acid has been developed and validated according to the following parameters: specificity, linearity, accuracy, repeatability, intermediate precision. The linearity was in the concentration range of $0.002-0.02$ mol L^{-1} ($r^2 = 0.9993$). The percentage of recovery was found to be in the range from 95.38 to 105.00 %. The values of %*RSD* for repeatability and intermediate precision were 1.86 and 1.95 %, respectively. The method is accurate and reliable, with the relative standard deviation of less than 2 %. It has been proven that the method developed is express, rapid, highly sensitive, accurate and sufficiently reliable.

Keywords: antioxidant activity; potentiometric method; validation; ascorbic acid

О. Ю. Маслов, С. В. Колісник, М. А. Комісаренко, Е. Ю. Ахмедов, А. О. Коваль, Т. А. Костіна,
О. В. Колісник

Національний фармацевтичний університет Міністерства охорони здоров'я України,
вул. Пушкінська, 53, м. Харків, 61002, Україна

Метрологічна характеристика розробленої методики визначення антиоксидантної активності аскорбінової кислоти

Анотація

Потенціометричну методику визначення антиоксидантної активності аскорбінової кислоти було розроблено і валідовано відповідно до таких параметрів: специфічність, лінійність, точність, прецизійність, внутрішня прецизійність. Лінійність зберігалася в діапазоні концентрацій $0,002-0,02$ моль л$^{-1}$ ($r^2 = 0,9993$). Визначено, що відсоток відновлення становить $95,38-105,00$ %, прецизійність та внутрішня прецизійність — 1,86 % та 1,95 %, відповідно. Методика характеризується як точна і надійна, має відносне стандартне відхилення менше 2 %. Доведено, що розроблена методика експресна, проста, високочутлива, точна і достатньо надійна.

Ключові слова: антиоксидантна активність; потенціометричний метод; валідація; аскорбінова кислота

Conflict of interests: The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

■ Introduction

Reactive oxygen species are generated endogenously in the mitochondrial oxidative phosphorylation, or they may arise from exogenous sources, such as xenobiotic compounds [1, 2]. Oxidative stress results in indirect or direct damage of nucleic acids, proteins, and lipid, and has been implicated in carcinogenesis, neurodegeneration, atherosclerosis, diabetes and aging [3, 4].

Ascorbic acid is a six-carbon lactone obtained from glucose in the liver. Human is not able to synthesize it due to the lack of *L*-gulonolactone oxidase [5]. Therefore, vitamin C must be obtained from the diet to maintain a normal metabolic functioning of the body. In the body of a human, vitamin C reacts with free radicals as an antioxidant [6]. The ascorbyl radical is formed when ascorbic acid loses one electron, the second form is called dehydroascorbic acid formed with losing an electron by the ascorbyl radical [7, 8].

Most of the physiological functions of vitamin C are related to its reduction properties [9, 10]. Ascorbic acid is involved in the synthesis of collagen, carnitine and neurotransmitters. In addition, ascorbic acid accelerates the healing process, affects the synthesis of a number of hormones, regulates hematopoiesis and normalizes capillary permeability [11–13].

According to Pubmed and ScienceDirect databases, the number of publications dealing with the study of the antioxidant activity of different substances is growing steadily (**Figure 1**). Known assays for determining the antioxidant activity are based on the oxidation of the test sample with oxidizing agents of various nature. Oxidizing agents can be inorganic compounds – $K_3[Fe(CN)_6]$, H_2O_2, $KMnO_4$, and organic compounds – 2,2-diphenyl-1-picrylhydrazyl (in DPPH assay), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (in ABTS assay), Fe(III)-tripyridyltriazine (in FRAP assay) [14]. The antioxidant activity of ascorbic acid has been determined by different assays, such as DPPH [15], ABTS [16], FRAP [17], chemiluscent and potentiometric method [18].

S. Martinez et al. [19] applied the potentiometric assay in determining the antioxidant activity of wine. The potentiometric assay they use is based on titration of test samples with an electrogenerated chlorine. Chlorine has the ability to enter into various reactions (radical, redox, electrophilic substitution and addition to multiple bonds). Due to this, titration with chlorine allows to cover a wide spectrum of biologically active components possessing antioxidant properties. However, in our opinion, this method has some disadvantages. Firstly, in the assay the pH equals 2. At the same time, for studies of the antioxidant activity related to living organisms, the pH value should be maintained in the range of 7.2–7.4 since it is physiological. Secondly, chlorine is rather toxic compound.
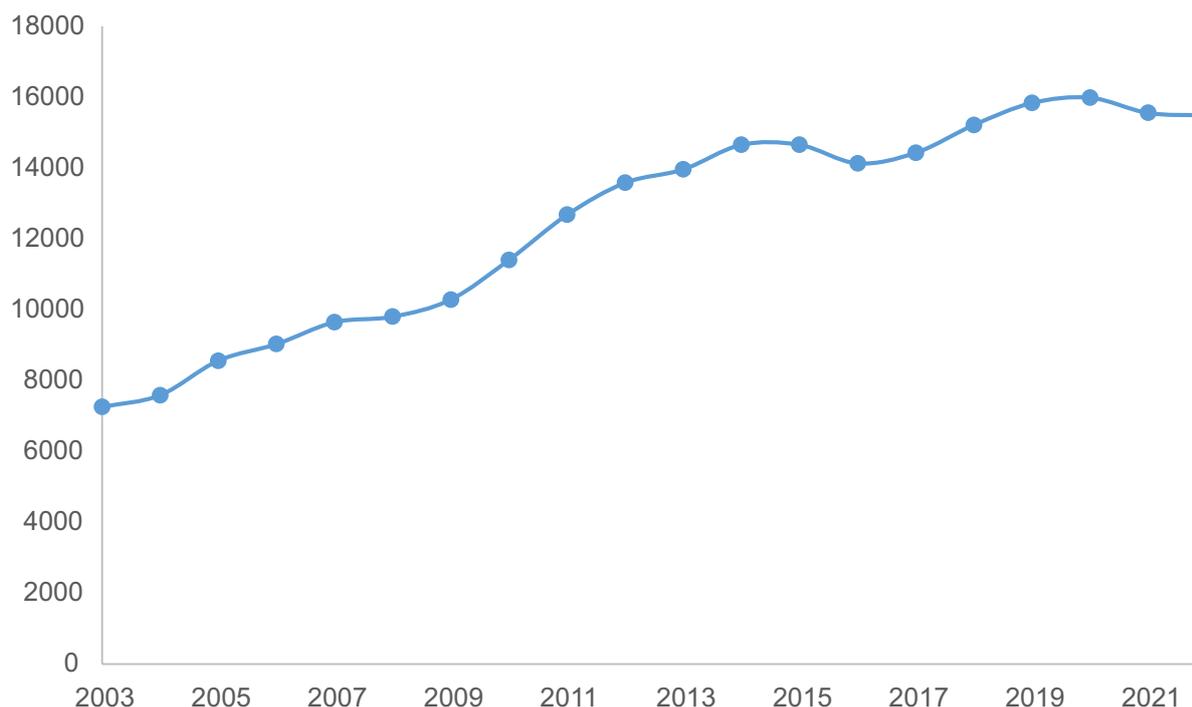
Figure 1. The number of published articles in the period of 2003 – 2022 years

Currently, ascorbic acid is found in numerous plants, e.g., rose hips, mountain ash, viburnum, currants, raspberries and citrus fruits. In addition, vitamin C is also an ordinary "guest" in caplets, tablets, capsules, drink mixes, multivitamin, antioxidant formulation and dietary supplements. Hence, the development of methods for determining the antioxidant activity of ascorbic acid is a promising task today. Thus, the aim of the present study was to develop and validate the potentiometric assay for determining the antioxidant activity of ascorbic acid.

■ Materials and methods

Reagents

Ascorbic acid ≥ 98.0% (Sigma-Aldrich). Reagents $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, $NaHPO_4$, KH_2PO_4 were of analytical grade and purchased from Reakhim (Kharkiv, Ukraine).

Instruments

A pH meter Hanna 2550 (FRG) with a combined platinum electrode EZDO 50 PO (Taiwan) were used during potentiometric measurements. The digital analytical balance AN100 (AXIS, Ukraine) with d = 0.0001 g was used for weighing.

Preparation of standard solutions

A stock solution of ascorbic acid (0.05 mol L^{-1}) was prepared by dissolving ascorbic acid in distilled water.

Model solutions of ascorbic acid were prepared by dilution of the stock solution of ascorbic acid (0.002, 0.006, 0.01, 0.014, 0.02 mol L^{-1}).

Preparation of phosphate buffer solutions

8.00 g of NaCl, 0.20 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 were dissolved in 800 mL of distilled water, and pH to 7.4 was adjusted with hydrochloric acid or sodium hydroxide, and the solution was diluted with distilled water to 1 L.

The procedure for the antioxidant activity determination

2 mmol L^{-1} solution of $K_3[Fe(CN)_6]$ was prepared by weighing 0.8232 g into a 25.0 mL volumetric flask, dissolving the compound in distilled water, and diluted to the volume with the same solvent. 0.02 mmol L^{-1} of $K_4[Fe(CN)_6]$ was prepared by weighing 0.0921 g into a 250.0 mL volumetric flask, dissolving the compound in distilled water, and diluted to the volume with the same solvent. Then 5.00 mL of the aliquot of both prepared solutions was taken and transferred into a 250.0 mL volumetric flask and diluted to the volume with 0.067 mmol L^{-1} phosphate buffer solution. 50.00 mL of the prepared intermediate solution was transferred into an electrochemical cell. The initial potential of the intermediate solution was measured after the initial one was determined, 1.00 mL of the aliquot of the solutions obtained was added, and a final potential was measured. The difference (ΔE) between the initial (E_0) and final (E_1) potentials was found [20].

The antioxidant activity was calculated according to the following equation and expressed as mmol L^{-1}:

$$AOA = \frac{C_{ox} - \alpha \cdot C_{red}}{1 + \alpha} \cdot K_{dil} \cdot 10^3$$

where $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - Eethanol)nF/2.3RT}$; C_{ox} – is the concentration of $K_3[Fe(CN)_6]$, mol L^{-1}; C_{red} – is the concentration of $K_4[Fe(CN)_6]$, mol L^{-1}; $E_{ethanol} - 0.0546 \times C_{\%} - 0.0091$; $C_{\%}$ – is the concentration of ethanol; ΔE – is the change of the potential; $F = 96485.333$ C mol^{-1} – is the Faraday constant; n = 1 – is the number of electrons in the electrode reaction; $R = 8.314$ J mol^{-1} K^{-1} – is the universal gas constant; T – 298 K; K_{dil} – is the coefficient of dilution.

Validation method

Validation of the potentiometric method for determining the antioxidant activity of ascorbic acid was performed according to the International Conference on Harmonization (ICH) [21]. The method proposed was validated by the following parameters: specificity, linearity, accuracy, repeatability and intermediate precision.

The specificity of the method was studied by the potentiometric titration of the solvent.

The linearity of the method was studied at 5 concentration levels (0.002, 0.006, 0.01, 0.014, 0.02 mol L^{-1}). The antioxidant activity was evaluated by the potentiometric method. The linearity was determined by a linear relationship between the logarithm concentrations of the ascorbic acid solutions prepared. The linear regression was calculated by the method of least squares to obtain the regression equation and determine the correlation coefficient (r^2). According to the requirements of ICH, the value of the correlation coefficient when studying the linearity should not exceed 0.999.

The accuracy was evaluated by calculating recovery of ascorbic acid using the standard addition method. Three levels of ascorbic acid concentration corresponding to 50, 100, 200% of the working concentration of ascorbic acid 0.1 mol L^{-1}

were taken. The acceptance criteria were *RSD* and should not exceed 2%.

The precision of the method was assessed by repeatability and intermediate precision. In the case of repeatability, the working solution of ascorbic acid with the concentration of 0.01 mol L^{-1} was analyzed six times at the same day. The intermediate precision was evaluated with the same concentration of ascorbic acid at different days. The acceptance criteria were *RSD* and should not exceed 2%.

The statistical processing of the experimental data obtained was performed in accordance with the monograph "Statistical analysis of the results of a chemical experiment" of the State Pharmacopeia of Ukraine.

■ Results and discussion

The potentiometric method for determining the antioxidant activity is based on the interaction of a mediator system with an antioxidant. The mediator system consists of an oxidizing agent and a reducing agent. When an antioxidant is added to the mediator system, the ratio of the oxidizing agent and reducing agent changes, which leads to a shift in the potential of the electrochemical cell. After that, the potential difference and the antioxidant activity of the antioxidant studied are calculated.

In order to develop a potentiometric assay for determining the antioxidant activity of ascorbic acid, it was necessary:

1) to choose the optimal redox electrode;

2) to choose the optimal ratio of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$;

3) the potential of the platinum electrode should correspond to the Nernst dependence of the potential change.

The main criteria for choosing the redox electrode are the range of measuring the potential of the electrochemical cell and the time of establishing the potential.

The potential of the electrochemical cell is measured by platinum, gold and glass-carbon electrodes. Thus, to choose the optimal electrode we analyzed characteristics of three electrodes mentioned above.

Table 1 shows that the glass-carbon electrode has the widest range of measuring the potential of the electrochemical cell, followed by the platinum electrode, whereas the gold one has the narrowest measuring potential of the electrochemical cell. Therefore, the platinum and glass-carbon electrodes meet the requirement of the range of

Table 1. Measuring ranges of potentials of electrodes

No.	Electrode	Measuring ranges, *V*
1	Platinum electrode	from -0.1 to +0.9
2	Gold electrode	from -0.1 to +0.3
3	Glass-carbon electrode	from -0.9 to +0.8

Table 2. The time of the establishing the potential of the electrochemical cell

No.	Electrode	Time, *sec*
1	Platinum electrode	10±0.3
2	Gold electrode	1800±36.0
3	Glass-carbon electrode	300±6.0

Note: n = 5, p < 0.05

measuring the potential of the electrochemical cell.

Table 2 demonstrates that the platinum electrode is the fastest (10 sec) in establishing the potential of the electrochemical cell, followed by the glass-carbon electrode (300 sec), and the gold electrode has the longest time (1800 sec).

According to the results obtained, the platinum electrode met all the requirements to the redox electrode and was the most suitable for determining the antioxidant activity of ascorbic acid. Thus, it was used in the study.

The accuracy of determining the redox potential of the system depends on how the Nernst dependence follows the potential change. **Figure 2** shows the dependence of the potential of the platinum electrode EZDO 50 PO in the sodium phosphate buffer with the pH 7.4 and various concentrations of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The pre-logarithmic coefficient in this dependence is 58.5 mV (**Figure 2**), which is close to the theoretical value of RT/nF = 59.16 mV in the Nernst equation for a one-electron process at 25°C. From mentioned above, it can be concluded that the platinum electrode is completely suitable according to the criteria set.

To select the optimal concentration and ratio of $K_3[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$, the following criteria were used:

1) the minimum change in the system potential must be at least 20 mV;

2) the high speed of equilibration;

3) the potential of the system must be stable over time.

Table 3 demonstrates the change in the potentials of the system with different ratios of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ concentrations after the introduction of the test samples (1.0–5.0 mol L^{-1} of ascorbic acid). It is easy to see that in the
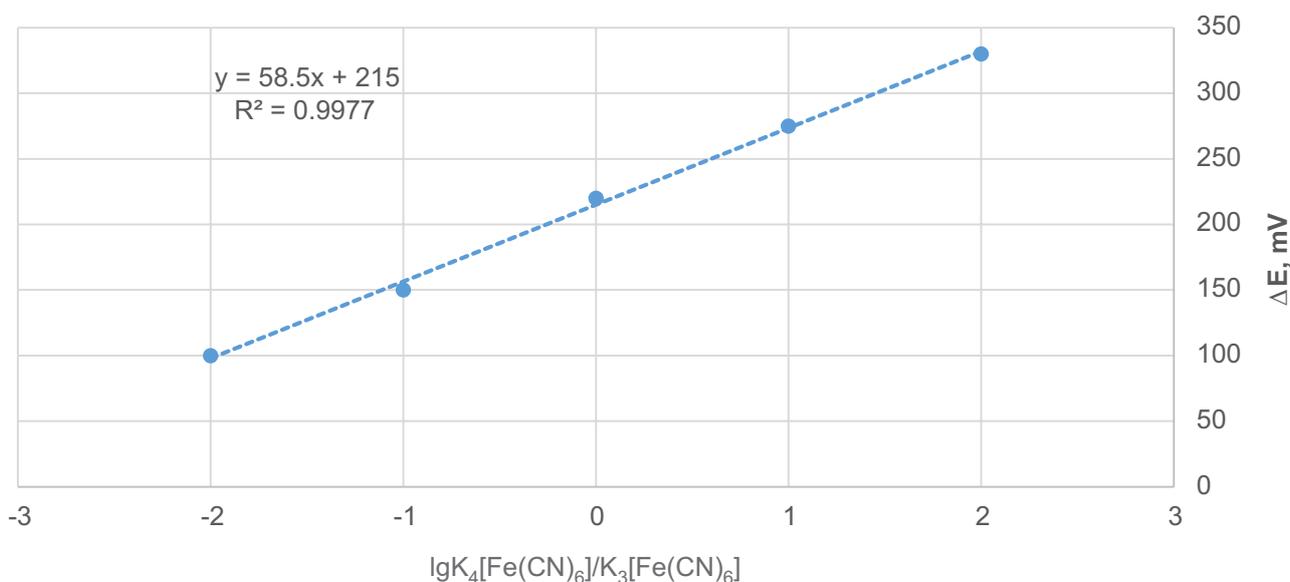
Figure 2. The dependence of the potential of the platinum electrode EZDO 50 PO in the sodium phosphate buffer with the pH 7.4 and various concentrations of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

Table 3. The change in the potential after the introduction of ascorbic acid (1.0 and 5.0 molL^{-1}) into the mediator system of various compositions

Mediator system	$K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, mol L^{-1}	ΔE for concentration of 1.0 mol L^{-1}, mV	ΔE for concentration of 5.0 mol L^{-1}, mV
System 1	0.002/0.00002	25.0±0.3	40.0±0.3
System 2	0.001/0.00001	40.0±0.3	100.0±0.3
System 3	0.1/0.001	2.0±0.3	12.0±0.3
System 4	0.05/0.001	10.0±0.3	25.0±0.3

Note: $n = 5$, $p < 0.05$

system 1 and 2 the minimum potential change is greater than 20 mV. However, the redox potential of system 2 is not stable for 30 minutes, this may be due to the fact that the concentration of $K_4[Fe(CN)_6]$ is low enough for the potential of the system to be stable over time. System 1 turned out to be the most stable over time, and its potential did not change over the course of an hour. Therefore, this mediator system was chosen to determine the antioxidant activity of the samples under study.

The linearity was proven in the concentration range from 0.002 to 0.02 mol L^{-1}. The regression equation of the curve had the following form: y = 2.5896x + 7.4011. The value of the correlation coefficient (r^2) was equal to 0.9993 (**Figure 3**).

The accuracy of the method was assessed by the percentage of recovery. The percentage of recovery was found to be in the range from 95.38 to 105.00% (**Table 4**).

The precision of the method was confirmed by repeatability and intermediate precision. The values

Table 4. Recovery studies using the standard method of addition

AO	AOA, mmol-eq. L^{-1}	Amount added of AO, mmol-eq.L^{-1}	Amount taken of AO (C_1), mmol-eq.L^{-1}	Amount recovered (C_2), mmol-eq.L^{-1}	%, Recovery $R = \dfrac{C_2}{C_1} \cdot 100\,\%$
Ascorbic acid	1.95	0.98	2.93	3.00	102.79
				3.02	103.07
				2.80	95.56
		1.95	3.90	4.00	102.39
				4.11	105.00
				3.72	95.38
		2.93	4.88	5.00	102.46
				5.14	105.00
				4.80	98.36
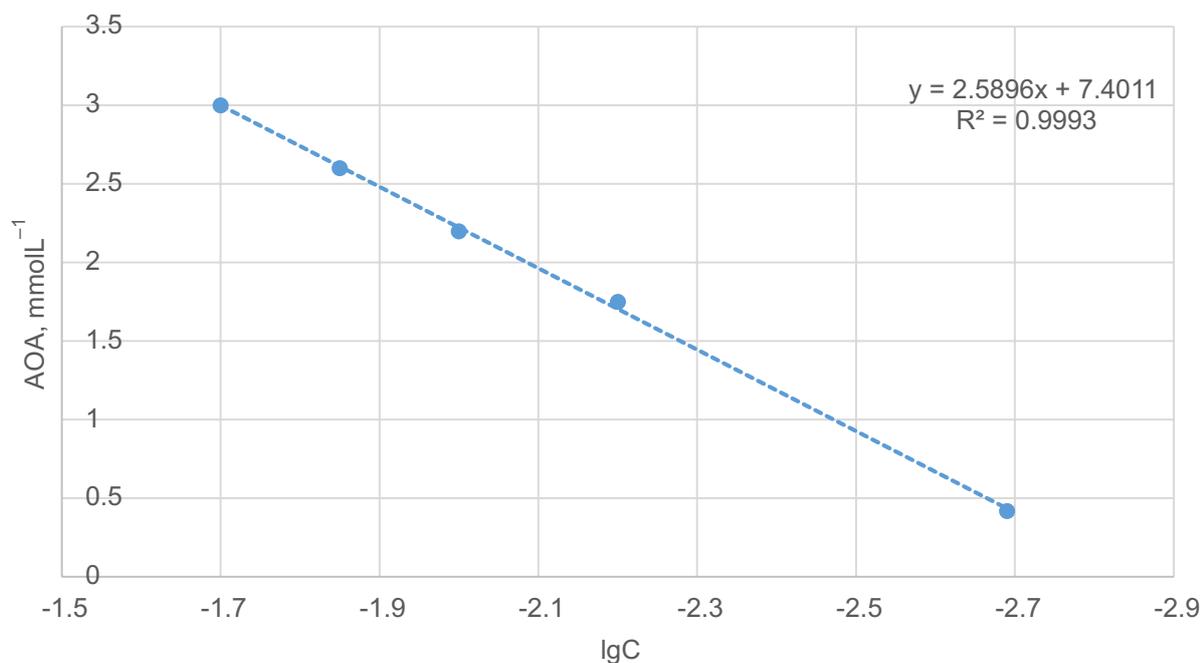
Figure 3. The calibration curve of the concentration logarithm *vs* the antioxidant activity of ascorbic acid

Table 5. Repeatability results for determining the antioxidant activity of ascorbic acid

Number of samples	AOA of ascorbic, mmol-eq. L^{-1}
1	1.95
2	1.99
3	1.91
4	1.91
5	2.00
6	1.95
Mean, mmol-eq. L^{-1}	1.95
SD	0.04045
Confidence interval, mmol-eq. L^{-1}	0.04
RSD	1.95

Table 6. Intermediate precision results for determining the antioxidant activity of ascorbic acid

Number of samples	Ascorbic acid	
	The first day	The second day
1	1.95	1.99
2	1.95	1.98
3	1.97	2.05
4	2.02	1.95
5	1.90	1.97
6	1.98	1.98
Mean, mmol-eq. L^{-1}	1.96	1.99
SD	0.03971	0.03386
Confidence interval, mmol-eq. L^{-1}	0.04	0.04
RSD, %	2.00	1.70
Mean of *RSD*, %	1.86	

of %*RSD* for repeatability and intermediate precision were 1.95 and 1.86%, respectively. The %*RSD* values were less than 2%. It proves that the method is precise (**Tables 5** and **6**).

■ Conclusions

The potentiometric assay for determining the antioxidant activity of ascorbic acid has been developed and validated according to the following parameters: specificity, linearity, accuracy, repeatability, intermediate precision. The method proposed can be used for routine analysis to determine the antioxidant activity of different objects under research and quality control purposes since the method developed is express, rapid, highly sensitive, accurate and sufficiently reliable.

■ References

1. Ray, P. D.; Huang, B.-W.; Tsuji, Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell. Signal.* **2012**, *24* (5), 981–990. https://doi.org/10.1016/j.cellsig.2012.01.008.
2. Andrisic, L.; Dudzik, D.; Barbas, C.; Milkovic, L.; Grune, T.; Zarkovic, N. Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer. *Redox Biol.* **2018**, *14*, 47–58. https://doi.org/10.1016/j.redox.2017.08.009.
3. Cioffi, F.; Adam, R. H. I.; Broersen, K. Molecular Mechanisms and Genetics of Oxidative Stress in Alzheimer's Disease. *J. Alzheimer's Dis.* **2019**, *72* (4), 981–1017. https://doi.org/10.3233/jad-190863.

4. Li, S.; Hong, M.; Tan, H.-Y.; Wang, N.; Feng, Y. Insights into the Role and Interdependence of Oxidative Stress and Inflammation in Liver Diseases. *Oxidative Med. Cell. Longev.* **2016**, *2016*, 1–21. https://doi.org/10.1155/2016/4234061.

5. Chang, S. K.; Ismail, A.; Daud, Z. A. M., Ascorbic Acid: Properties, Determination and Uses. In *Encyclopedia of Food and Health*, Caballero, B.; Finglas, P. M.; Toldrá, F., Eds. Academic Press: Oxford, 2016; pp 275–284. https://doi.org/10.1016/B978-0-12-384947-2.00044-1.

6. Tamari, Y.; Nawata, H.; Inoue, E.; Yoshimura, A.; Yoshii, H.; Kashino, G.; Seki, M.; Enomoto, T.; Watanabe, M.; Tano, K. Protective roles of ascorbic acid in oxidative stress induced by depletion of superoxide dismutase in vertebrate cells. *Free Radic. Res.* **2012**, *47* (1), 1–7. https://doi.org/10.3109/10715762.2012.734916.

7. Gioia, M. G.; Andreatta, P.; Boschetti, S.; Gatti, R. Development and validation of a liquid chromatographic method for the determination of ascorbic acid, dehydroascorbic acid and acetaminophen in pharmaceuticals. *J. Pharm. Biomed. Anal.* **2008**, *48* (2), 331–339. https://doi.org/10.1016/j.jpba.2008.01.026.

8. *Handbook of vitamins*, 4th Ed. Rucker, R. B.; Zempleni, J.; Suttie, J. W.; McCormick, D. B., Eds. CRC Press: Boca Raton, 2012. https://doi.org/10.1201/9781420005806.

9. Griffiths, H. R.; Lunec, J. Ascorbic acid in the 21st century – more than a simple antioxidant. *Environ. Toxicol. Pharmacol.* **2001**, *10* (4), 173–182. https://doi.org/10.1016/s1382-6689(01)00081-3.

10. Smirnoff, N., Chapter 4 – Vitamin C: The Metabolism and Functions of Ascorbic Acid in Plants. In *Advances in Botanical Research*, Rébeillé, F.; Douce, R., Eds. Academic Press: 2011; Vol. 59, pp 107–177.

11. Carr, A.; Maggini, S. Vitamin C and Immune Function. *Nutrients* **2017**, *9* (11), 1211. https://doi.org/10.3390/nu9111211.

12. Ashino, H.; Shimamura, M.; Nakajima, H.; Dombou, M.; Kawanaka, S.; Oikawa, T.; Iwaguchi, T.; Kawashima, S. Novel Function of Ascorbic Acid as an Angiostatic Factor. *Angiogenesis* **2003**, *6* (4), 259–269. https://doi.org/10.1023/b:agen.0000029390.09354.f8.

13. Arrigoni, O.; De Tullio, M. C., Ascorbic acid: much more than just an antioxidant. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2002**, *1569* (1), 1–9. https://doi.org/10.1016/S0304-4165(01)00235-5.

14. *Measurement of Antioxidant Activity and Capacity*. Apak, R.; Capanoglu, E.; Shahidi, F., Eds. John Wiley & Sons: New York, 2017.

15. Chen, Z.; Bertin, R.; Froldi, G. EC$_{50}$ estimation of antioxidant activity in DPPH assay using several statistical programs. *Food Chem.* **2013**, *138* (1), 414–420. https://doi.org/10.1016/j.foodchem.2012.11.001.

16. Jabłońska-Ryś, E.; Zalewska-Korona, M.; Kalbarczyk, J. Antioxidant capacity, ascorbic acid and phenolics content in wild edible fruits. *Journal of Fruit and Ornamental Plant Research* **2009**, *17* (2), 115–120.

17. Barba, F. J.; Esteve, M. J.; Tedeschi, P.; Brandolini, V.; Frígola, A. A Comparative Study of the Analysis of Antioxidant Activities of Liquid Foods Employing Spectrophotometric, Fluorometric, and Chemiluminescent Methods. *Food Anal. Methods* **2012**, *6* (1), 317–327. https://doi.org/10.1007/s12161-012-9441-3.

18. Maslov, O. Y.; Kolisnyk, S. V.; Ponomarenko, S. V.; Ahmedov, E. Y. O.; Shovkova, Z. V. The study of the effect of ethyl alcohol concentrations on the antioxidant activity of ascorbic acid solutions. *J. Org. Pharm. Chem.* **2021**, *19* (2), 44–47. https://doi.org/10.24959/ophcj.21.231947.

19. Martinez, S.; Valek, L.; Piljac, J.; Metikoš-Huković, M. Determination of wine antioxidant capacity by derivative potentiometric titration with electrogenerated chlorine. *Eur. Food Res. Technol.* **2004**, *220* (5–6), 658–661. https://doi.org/10.1007/s00217-004-1072-5.

20. Maslov, O.; Kolisnyk, S.; Komisarenko, M.; Golik, M. Study of total antioxidant activity of green tea leaves (*Camellia sinensis* L.). *Herba Pol.* **2022**, *68* (1), 1–9. https://doi.org/10.2478/hepo-2022-0003.

21. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology Q2(R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf (accessed Aug 17, 2023).

Information about the authors:

Oleksandr Yu. Maslov (*corresponding author*), Ph.D. in Pharmacy, Teaching Assistant of the General Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0001-9256-0934; e-mail for correspondence: alexmaslov392@gmail.com; tel. +380 66 4257224.

Sergii V. Kolisnyk, D.Sc. in Pharmacy, Professor, Head of the General Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0002-4920-6064.

Mykola A. Komisarenko, Ph.D. in Pharmacy, Teaching Assistant of the Department of Pharmacognosy and Nutritiology, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0002-1161-8151.

Elshan Yu. Akhmedov, Ph.D. in Pharmacy, Associate Professor of the General Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0001-6727-8259.

Alla O. Koval, Ph.D. in Pharmacy, Associate Professor of the General Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0001-9491-0459.

Tatiana A. Kostina, Ph.D. in Pharmacy, Associate Professor of the General Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0001-5048-8575.

Olena V. Kolisnyk, Ph.D. in Pharmacy, Associate Professor of the Pharmaceutical Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0003-0558-3164.