ISSN 2078-5615. Вісник Львівського університету. Серія хімічна. 2023. Випуск 64. С. 185–197
Visnyk of the Lviv University. Series Chemistry. 2023. Issue 64. P. 185–197

Аналітична хімія

УДК 543.5+544.6

VALIDATION OF METHODS FOR THE DETERMINATION OF MEDICINAL SUBSTANCES USING THE EXAMPLE OF VOLTAMPEROMETRICAL DETERMINATION OF ATROPINE IN EYE DROPS AND SOLUTIONS FOR INJECTIONS

O. Dushna*, L. Dubenska

Ivan Franko National University of Lviv,
Kyryla i Mefodiya Str., 6, 79005 Lviv, Ukraine
e-mail: olha.dushna@lnu.edu.ua

Validation is an important component of the procedures that a laboratory must perform before implementing a new method of drug analysis. By the requirements of the European Pharmacopoeia and the State Pharmacopoeia of Ukraine, it is necessary to determine the following analytical characteristics for the analytical method: specificity, robustness, linearity, accuracy, precision, and their components.

This work gives a short validation report of the proposed voltammetric method for determining atropine in eye drops and solutions for injections. The planar electrochemical cell with the working boron-doped diamond electrode was used. The developed method was validated according to the criteria of uncertainty, linearity, accuracy, and precision.

Keywords: validation, ISO/IEC 17025, electrochemical methods, voltammetry, atropine.

DOI: https://doi.org/10.30970/vch.6401.185

1. Introduction

Method validation is an important requirement in the practice of chemical analysis. Most analytical chemists are aware of its importance, but why it should be done and when, and exactly what needs to be done, is not always clear to them [1]. The following regulatory documents explain the term "validation": ISO/IEC 17025 [2], ISO 15195 [3], ISO 9000 [4], Vocabulary of Metrology (VIM) 3 [5] та VIM 4 [6]. In Ukraine, these regulatory documents have been harmonized and accepted as national: DSTU ISO/IEC 17025 [7], DSTU ISO 15195 [8], and DSTU ISO 9000 [9], respectively. In particular, ISO/IEC 17025 states that validation – confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. According to VIM 3 and VIM 4, the provision of objective evidence that a given item fulfills specified requirements is called verification. Therefore, these two concepts are close, but they need to be distinguished. Thus, validation is a verification (check) that a certain object, for example, a measurement method or a measurement tool, is suitable for achieving the set measurement goal.

Certain technical requirements must be taken into account when developing an analytical method and its validation. These requirements are usually described in regulatory

documents and recommendations. For example, for the development of methods of quality control of medicinal products used in humanitarian medicine, the guideline is used "Validation of Analytical Procedures" prepared by the International Conference on Harmonization of Technical Requirements for Registration Pharmaceuticals for Human use (ICH) [10–12].

Validation is very closely related to method development: many of the method characteristics that are determined during validation are usually evaluated during method development (Fig. 1). In Ukraine, the method is usually validated according to the criteria according to the recommendations of the State Pharmacopoeia of Ukraine [13], European Pharmacopoeia [14], the United States Pharmacopeia [15], and ICH guidelines [10-12]. And they also take into account the recommendations of the directives of the European Union. For example, in the development and validation of methods for controlling residual amounts of organic substances, the validation criteria are described in Directive EC 2002/657/EC [16] and Regulation (EU) 2021/808 [17]. Table 1 shows a comparison of the validation criteria that are given in the State Pharmacopoeia of Ukraine and ICH guidelines for the control of active substances in drugs.

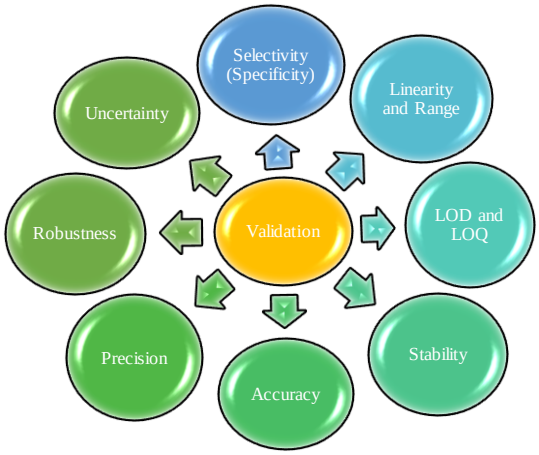


Fig. 1. Criteria that are typically evaluated during method validation

Regarding the control of residual amounts, the following validation criteria are mandatory for these methods: limit of detection (LOD), limit of quantification (LOQ), trueness or accuracy, precision, selectivity and specificity, applicability/ruggedness, stability [16–17]. Also, when validating such methods, two approaches are used: a) approach within the limits of one laboratory; b) an approach using interlaboratory studies that are established Codex Alimentarius, ISO or the IUPAC [18].

Table 1

Data elements required for analytical validation according to ICH [10-12] and the State Pharmacopoeia of Ukraine [13].

Necessary validation parameters for analytical studies	ICH Guidelines [10–12]	The State Pharmacopoeia of Ukraine [13]
Selectivity (*Селективність*)* Specificity (*Специфічність*)	+	+
Linearity and Range (*Лінійність*)	+	+
LOD (*Межа виявлення*)	+	+
LOQ (*Межа визначення*)	+	+
Accuracy (*Правильність*)		+
Precision (*Прецизійність*)		
-repeatability (*Збіжність/Повторюваність*)	+	+
-intermediate precision (within day precision) (*Проміжна прецизійність*)	+	+
-reproducibility (between day precision) (*Відтворюваність*)	+	+
Robustness (*Робасність*)	Recommended	+
Ruggedness (*Стабільність*)	Recommended	–
Sensitivity (*Чутливість*)	Recommended	–

**Terms are indicated in Ukrainian.*

Specificity and Selectivity

Specificity and selectivity related to the detection and selectivity of the determination of the active substance. The selectivity of the method is usually described as the ability to determine the substance in the presence of extraneous substances, as well as under the influence of the sample matrix. In most cases, this is the first parameter that is considered when developing a method [19].

Linearity and Range

Linearity is a necessary and indispensable parameter for quantification. The linearity of the analytical method is a measure of the extent to which the dependence of the analytical signal on the concentration of the active substance is a straight line. Measure the analytical signal for standard solutions of at least six different concentrations. At least three repeated measurements are performed for each point. Usually, the data of the calibration graph are processed using the method of least squares, the regression analysis.

According to this method, it is possible to obtain the slope of the calibration curve (*b*), the y-intercept (*a*), the standard deviation of each of the parameters, as well as the correlation coefficient (*R*) or the coefficient of determination (R^2). All these parameters are basic information about the linearity of the method. For most methods, the correlation or determination coefficient should exceed 0.998 [20–22].

LOD and LOQ

The limit of detection (LOD, DL, in EU directives [16] CCβ) is the smallest amount or concentration of a component that can still be detected using a certain method with a given reliable probability *P* or with a reliability factor chosen according to the reliable probability *P*. The following approaches are used to calculate the LOD: visual evaluation method, and signal-to-noise ratio method, based on the calculation using the standard [10].

These types of LOD calculations are recommended by the official guidelines and pharmacopeias.

The limit of quantification (LOQ, less often limit of determination, C_{lim}, in EU directives [16] CCα) is the smallest amount or concentration of a component that can be reliably determined by a particular technique with a given probability *P*. In most cases, LOQ is taken to be the smallest concentration that can be determined with a relative standard deviation of $S_r \leq 0.33$ [23]. The estimation of the LOQ is mostly based on the preliminary estimation of the LOD.

Thus, it has been proven that, provided the grading function is linear and the values of the standard deviations of the background signal and the minimum analytical signal are close, the LOQ exceeds the LOD by 2–3 times. The IUPAC Recommendations [24] also default to a factor of 10, and if the standard deviation is approximately the same for low concentrations, then a factor of 10 corresponds to a relative standard deviation of RSD = 10 %. We should add that in VIM 3 the concept of LOQ is not defined.

Stability

The criterion of stability describes any possible degradation or destruction of analytes during the entire process of analysis – sampling, storage, preparation, analysis, etc. The stability of solutions and reagents of the analysis is evaluated over a certain period of time, as well as relative to the temperature of the environment. The values of the relative standard deviation (RSD) are calculated to assess the criterion of stability [25].

Accuracy

The accuracy of the method is assessed by the closeness of the average value of the results of multiple measurements to the true value of the measuring substance. The value specified in the certificate or the result obtained by an independent (reference) method is usually taken as the true value. Certified reference material (CRM) are also often used for the true value. Certified reference material is a generally recognized means of establishing metrological traceability.

A practical estimate of the accuracy can be obtained, which is usually the bias. The practical determination of the bias consists in comparing the average value of the results obtained using the designed method with the true value [10–12].

Precision

The precision criterion covers parameters of repeatability and reproducibility.

Repeatability is a characteristic that reflects the closeness of results obtained repeatedly by the same means, by the same method, under the same conditions, and with the same thoroughness over a short period of time. In other words, this is the closeness of parallel results. Repeatability is related to random measurement error. Values of standard deviation (SD) and the relative standard deviation (RSD) can be used to quantify the assessment of repeatability.

Precision is also characterized as the closeness between measured values of quantities obtained during repeated measurement on the same or similar objects under certain regulated conditions. Measurements are performed on samples of the same material using the same method but over a long period of time. It can also be done by different performers of the analysis using the same type, but different equipment.

Note that terms are sometimes used to denote precision: "intermediate precision" (within-day precision), "intra-laboratory precision" and "intra-laboratory reproducibility".

Reproducibility indicates the closeness of measurement results obtained in different laboratories [10–12].

Robustness

Robustness is the stability of the analytical method. That is, the ability to preserve its characteristics in the presence of small, but deliberate changes in the conditions and parameters of the method. For example, change in the pH values of the solution, and the temperature of the analysis. According to the results of such an experiment, it is possible to determine the variable parameters of the method, which have the greatest influence on the signal of the substance to be determined. This implies that the parameters that significantly affect the analytical signal must be carefully (severely) controlled during the application of the method [10–12].

Uncertainty

The uncertainty of a result is a parameter that describes a range within which the value of the quantity being measured is expected to lie, taking into account all sources of error [26]. Two approaches are used to estimate uncertainty: bottom-up uncertainty evaluation та top-down uncertainty evaluations [27].

The letter "u" denotes the uncertainty. However, there are different forms of presenting uncertainty:

– $u(x_i)$ – the standard uncertainty of the quantity measurement x_i – is the uncertainty expressed as a standard deviation;

– $u_c(y)$ – the complete standard uncertainty of measurement – is a mathematical combination of several individual standard uncertainties of measurement;

– U – extended measurement uncertainty is the characteristic that the laboratory usually provides to the customer. The extended measurement uncertainty characterizes the interval within which the value of the measured value can lie with a greater probability [28].

According to the recommendations of the State Pharmacopoeia of Ukraine [13], and European Pharmacopoeia [14], the complete uncertainty denotes Δ_{As}. In accordance with [29–30] it is calculated taking into account a tolerance of B = 5 %.

$$\Delta_{As} \leq B \cdot 0.32. \quad (1)$$

The complete uncertainty of the analysis results consists of the uncertainty of the sample preparation and the uncertainty of the final analytical operation:

$$\Delta_{As} = \sqrt{(\Delta_{SP})^2 + (\Delta_{FAO})^2}. \quad (2)$$

The uncertainty of sample preparation Δ_{SP} was calculated based on the method of sample preparation by the formula:

$$\Delta_{SP} = \sqrt{\sum_i \Delta_i^2}, \quad (3)$$

where Δ_i are the individual components of uncertainty according to [13].

The uncertainty of the final analytical operation Δ_{FAO} was calculated by the formula:

$$\Delta_{FAO} = 1.65 \cdot \sqrt{\frac{2 \cdot s_I^2}{3}}, \quad (4)$$

where S_I – the uncertainty of measuring the analytical signal (according to the passport of the device).

In this work, we provide a short report of validation according to the recommendations of the State Pharmacopoeia of Ukraine for the voltammetric method of determining atropine using a working electrode – boron-doped diamond electrode (BDDE). Previously, we have developed a method of voltammetric determination of atropine using the planar electrochemical cell with the working electrode – BDDE with different diameters of electode (1, 2 and 3 mm). Atropine is oxidized forming one peak at the potential +1.5 V in 2 M $HClO_4$ using cyclic voltammetry, characterized by being diffusion-controlled and irreversible process [31]. The development method was tested during the analysis of solutions for injection and eye drops. The concentration of atropine of solution for injection and eye drops was determined according to calibration graph and to the standard addition technique [31]. Validation of the method was carried out using an electrode with a diameter of 2 mm. The validation of the developed method was carried out according to the following criteria: uncertainty, accuracy, linearity and precision.

2. Materials and experimental procedures

2.1. Reagents

The substance of atropine sulfate (CAS 5908-99-6) with content of active substance 99 % was purchased from Sigma-Aldrich. Stock standard solution (SSS) with atropine concentration $1.0 \cdot 10^{-3}$ M was prepared as follows: the exact amount of atropine substance was dissolved in highly purified double-distilled water in a 25 mL volumetric flask, the volume was brought to the mark, and the solution was mixed thoroughly. SSS was stable during two weeks when stored in the fridge.

The inorganic acid 2 M $HClO_4$ was used in this work as supporting electrolyte.

2.2. Apparatus

All voltammetric measurements were performed using POL-20 digital device (MThech Lab, Ukraine). The accuracy of potential measurements was 0.1 mV. The uncertainty of the current measurement did not exceed 1 % [32].

The three-electrode cell system was used with a graphite electrode as the counter electrode and Ag/AgCl/ 3 M KCl as the reference electrode. A highly boron-doped BDDE with a diameter electrode of 2 mm was applied as a working electrode. The detailed characteristics of the electrode are given in [31, 33–34]. At the beginning of every work day, the surface of BDD electrode was rinsed with highly purified double-distilled water and anodically pretreatment at + 2.0 B in 0.5 M H_2SO_4 for 120 seconds to clean its surface followed by cathodic pretreated by applying – 2.0 B for 120 seconds.

The voltammograms were recorded using differential pulse voltammetry (DPV) in the range from 0 to 2.0 V. Received data were statistically analyzed by Origin 2018 (OriginLab, USA). All voltammetric measurements were performed in triplicate (n = 3) at room temperature.

2.3. Sample Preparation

Chemical glassware (flasks and pipettes) of class A were used in the work.

Preparation of working solution SSS of atropine. Nine model solutions were prepared by dilution with SSS in the concentration range from 8 to 12 μM, which corresponds to the limits of the application range of the technique (from 80 % to 120 % relative to the nominal content of atropine in pharmaceutical preparations) [13, 29–30].

In parallel, a standard solution of atropine (C_{STD}) with concentration 10 μM was prepared for comparison.

All prepared solutions were analyzed as follows: the aliquots of the prepared solution (100 μL) were dropped by a micropipette on the sensitive part of the planar electrochemical cell covering all three electrodes and the voltammograms were recorded.

Validation of the method was carried out for solutions for the injection "Atropine-Darnitsa" (Pharmaceutical Company Darnitsa) and "Atropine Sulfate" (GNCLS Experimental Plant Ltd) contain of atropine 1.0 mg per 1 mL. Eye drops "Atropine Sulfate"(GNCLS Experimental Plant Ltd) contain of atropine 10 mg per 1 mL. All these drugs are made in Ukraine and purchased in a local pharmacy.

Preparation of pharmaceuticals for voltammetric analysis. The procedure for preparing the solutions for the injection sample for voltammetric determination was as follows: a volume of 1 mL of the eye drops solution was taken from the bottle by a micropipette and diluted in double-distilled water in a 25 mL volumetric flask while bringing the volume to the mark, and mixing thoroughly. According to the regulatory documents of this drug, the concentration of this solution is $1.04 \cdot 10^{-3}$ M. The aliquots of the prepared solution were added to a 25 mL volumetric flask to obtain the desired concentration (for example 0.25 mL for $1.04 \cdot 10^{-5}$ M) and filled with supporting electrolyte (2 M $HClO_4$) to the mark.

The procedure of preparing the eye drops sample for voltammetric determination was as follows: a volume of 1 mL of a solution of eye drops was taken from the bottle by a micropipette and diluted with double-distilled water in a 250 mL volumetric flask while bringing the volume to the mark and mixing thoroughly. According to the regulatory documents of this drug, the concentration of this solution is $1.04 \cdot 10^{-3}$ M. The aliquots of the prepared solution were added to a 25 mL volumetric flask to obtain the desired concentration (for example 0.25 mL for $1.04 \cdot 10^{-5}$ M) and filled with supporting electrolyte (2 M $HClO_4$) to the mark.

3. Results and discussion

Uncertainty

The uncertainty was calculated according to the recommendations of the State Pharmacopoeia of Ukraine, according to formulas (1–4). The results are presented in Tables 2–3.

Table 2

Calculation uncertainty of sample preparation for the determination of atropine in pharmaceuticals

No.	Sample preparation	The parameter of the calculation formula	The uncertainty by [13, 29–30]	
			SSS	TSD
	solution for injection			
1	Selection of sample standard of atropine	m_0	0.15%	–
2	Pipette aliquot of 1mL selection	1	0.6 %	
3	Bringing up to volume in a volumetric flask of 25mL	25	0.23 %	
4	Pipette aliquot of 1mL selection	1	0.6 %	
5	Bringing up to volume in a volumetric flask of 25mL	25	0.23 %	
	eye drops			
5	Selection of sample standard of atropine	m_0	0.15 %	–
6	Pipette aliquot of 1mL selection	1	0.6 %	
7	Bringing up to volume in a volumetric flask of 250 mL	250	0.08 %	
8	Pipette aliquot of 1mL selection	1	0.6 %	
9	Bringing up to volume in a volumetric flask of 25mL	25	0.23%	

Table 3

Uncertainty components calculated according to the State Pharmacopoeia of Ukraine [13]

Uncertainty, %	Solution for injection	Eye drops
The uncertainty of the sample preparation	0.7013	0.8954
The uncertainty of the final analytical operation	0.08	
The total uncertainty	0.7059	0.8989

The calculated value of the total uncertainty of the results of analysis does not exceed the maximum permissible uncertainty of analysis. Moreover, it is indicated that the sample preparation and analytical measurements of the signal do not cause a significant error in the results of the analysis.

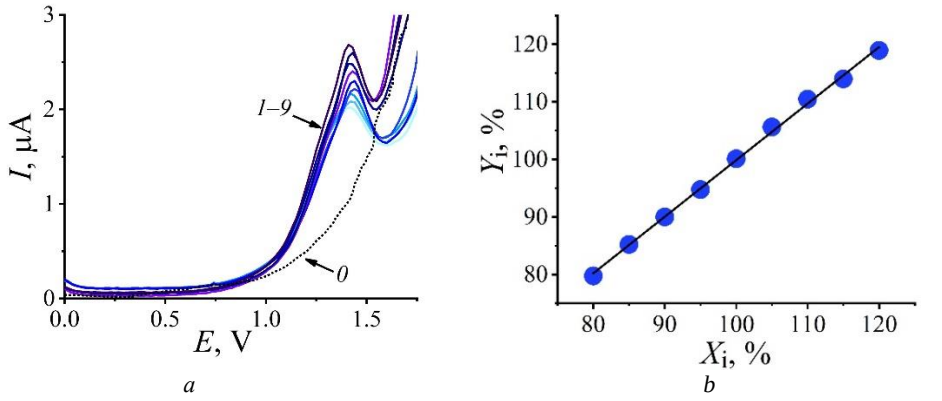
Linearity, accuracy, and precision

Nine model solutions in the concentration range from 8.0 to 12.0 μM were analyzed to study linearity, accuracy, and precision. The preparation of solutions is described in point 2.3. The results of the analysis of model solutions of atropine for pharmaceuticals are shown in Table 4. Figure 2 shows voltammograms and the graph of the dependence of current versus concentration of atropine in normalized coordinates.

Table 4

The results of the analysis of model solutions of atropine for pharmaceuticals

No. model solution	Introduced SSS *V*, mL	Concentration model solution *C*, μM	Introdused, $X_i = \frac{C_i}{C_{STD}} \cdot 100\%$	Current value *I*, μA	Found, $Y_i = \frac{I_i}{I_{STD}} \cdot 100\%$	Found in % to introduced $Z_i = \frac{Y_i}{X_i} \cdot 100$ %
1	0.80	8.0	80.00	1.679	79.76	99.70
2	0.85	8.5	85.00	1.793	85.20	100.23
3	0.90	9.0	90.00	1.894	90.00	100.00
4	0.95	9.5	95.00	1.994	94.75	99.74
5	1.00	10.0	100.00	2.107	100.09	100.09
6	1.05	10.5	105.00	2.210	105.61	100.58
7	1.10	11.0	110.00	2.328	110.46	100.42
8	1.15	11.5	115.00	2.399	113.96	99.09
9	1.20	12.0	120.00	2.504	118.93	99.11



a *b*

Fig. 2. The voltammograms (*a*) and the graph of the dependence of current versus concentration of atropine in normalized coordinates (*b*): *0* –the background line (without atropine); *1–9* – voltammograms in solutions of atropine in the concentration range from 8.0 to 12.0 μM

The Table 5 shows the parameters of linearity, accuracy and precision calculated by [29–30] for the determination of atropine in drugs.

Using the linearity parameters S_a and *b*, it is possible to estimate the limit of detection (LOD) and the limit of quantification (LOQ) according to the equations: $LOD = 3.3S_a/b$; $LOQ = 10S_a/b$.

For linearity in normalized coordinates, LOD and LOQ values are calculated as a percentage of the concentration of the comparison solution, which makes it possible to estimate a certain "margin of safety" of the procedure. Therefore,

$$LOD = 3.3 \cdot \frac{1.5073}{0.9825} = 5.063\ \% \text{ and } LOQ = 10 \cdot \frac{1.5073}{0.9825} = 15.34\ \%,$$

that significantly less than the lower concentration range (80 %), therefore, does not affect the accuracy of the analysis.

Table 5

Results of estimating validation criteria: the linearity, accuracy, and precision for the method determining atropine in drugs.

Parameter	Value	Critical values	Conclusion
Linearity			
Slope *b*	0.9825	0.975–1.025	Maintained
S_b	0.0149		
Intercept *a*	1.6156	2.6	Maintained
S_a	1.5073	–	
Residual standard deviation, S_0	0.5789	0.84	Maintained
The correlation coefficient, r	0.99919	–	
Criterion for the linear correlation coefficient, Rc	0.99908	0.99810	Maintained
Accuracy and precision			
Average value Z, %	99.89		
Relative standard deviation S_z, %	0.53		
Relative reliable interval $\Delta_{As}.\% = t(95\ \%, 8)\cdot S_z$	0.98	1.6	Maintained
Systematic error, δ	–0.115	0.51	Maintained

4. Conclusions

Validation of the method is a significant component of the development and implementation of the procedure in the work of the analytical laboratory. The number of validation criteria that the laboratory should investigate depends on the application and task of the developed method. According to ISO/IEC 17025, it is necessary to validate not only newly developed methods, but also standard/non-standard methods that were not included in the scope of the analytical laboratory. Validation criteria are established by normative documents (DSTU, ISO, directives of the European Union), and for the analysis of medicinal products, by Pharmacopoeias, in particular, the State Pharmacopoeia of Ukraine. The main validation criteria are selectivity, linearity, limits of detection and quantiation, accuracy and precision, and robustness.

In this work, we have provided a short validation report according to the recommendations of the State Pharmacopoeia of Ukraine for a new voltammetric method for determining atropine in eye drops and solutions for injections using a planar cell with a working electrode – BDDE. The results of the validation evaluation (criteria of linearity, accuracy, precision, and uncertainty) confirmed the correctness of the method.

1. *Magnusson B., Ornemark U.* et al. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics (2nd ed.) // Eurachem Guide. 2014. 63 p. www.eurachem.org.
2. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories, is the international reference for testing and calibration laboratories wanting to demonstrate their capacity to deliver reliable results.
3. ISO 15195:2018. Laboratory medicine – Requirements for the competence of calibratio laboratories using reference measurement procedures.
4. ISO 9000. International standards for quality management.
5. *Barwick V. J., Prichard E.* Eurachem Guide: Terminology in Analytical Measurement – Introduction to VIM 3 // Eurachem Guide. 2011. 38 p.
6. International Vocabulary of Metrology. Fourth edition – Committee Draft (VIM4 CD). https://iupac.org/international-vocabulary-of-metrology/
7. ДСТУ EN ISO/IEC 17025:2019 General requirements for the competence of testing and calibration laboratories (EN ISO/IEC 17025:2017, IDT; ISO/IEC 17025:2017, IDT).
8. ДСТУ EN ISO 15195:2015 Medical laboratories – Requirements for quality and competence (EN ISO 15195:2003, IDT).
9. ДСТУ ISO 9000:2015 Quality management systems – Fundamentals and vocabulary (ISO 9000:2015, IDT).
10. ICH Guideline Q2A (R1), Validation of Analytical Procedures; Text and methodology. November, 2005.
11. ICH Guideline Q2B, Validation of Analytical Procedures; Methodology. November, 1996.
12. *Ermer J., Miller J. H., McB., Eds.* Method Validation in Pharmaceutical Analysis. Wiley-VCH Publishers. Weinheim, 2005. 411 p.
13. The State Pharmacopoeia of Ukraine, Second edition in three volumes. Kharkiv, 2015. 1128 p. http://sphu.org/viddil-dfu
14. European Pharmacopoeia. 10-th Ed. Strasbourg: Council of Europe, 2021.
15. The United States Pharmacopeia USP 37.2. The National Formulary NF 32. Rockville: The United States Pharmacopeial Convention; 2016. 4501 p.
16. Directive EC 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results // Official Journal L 22. 2002. P. 0008–0036.
17. Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC // Official Journal L 180. 2021. P. 84–109.
18. IUPAC. Protocol for the design, conduct and interpretation of method-performance studies // Pure & AppliedChem. 1995. Vol. 67. 331 p.
19. *Danzer K.* Selectivity and specificity in analytical chemistry. General considerations and attempt of a definition and quantification // Fresenius' Journal of Analytical Chemistry. 2001. Vol. 369. P. 397–402. DOI: https://doi.org/10.1007/s002160000684

20. *Shabir G. A., Lough W. J., Arain S. A., Bradshow, T. K.* Evaluation and Application of Best Practice in Analytical Method Validation // J. Liq. Cromatogr. Related. Technol. 2007. Vol. 30. P. 311–333.
21. *Crowter J. B., Ahuja S., Scypinski S.* Validation of pharmaceutical methods // In Handbook of Modern Pharmaceutical Analysis. 2001. 555 p.
22. *Baumann K.* Regression and calibration for analytical separation techniques. Process Control and Quality. Process Control // Qual. 1997. Vol. 10. P. 75–112.
23. Eurachem: https://www.eurachem.org/
24. International Union of Pure and Applied Chemistry (IUPAC): www.iupac.org
25. *Grimm W.* Storage Conditions for Stability testing in the Ec, Japan and USA; the most important market for drug products // Drug Dev. Ind. Pharm. 1993. Vol. 19. P. 2795–2830.
26. *Gumustas M., A. Ozkan S.* The role of and the place of method validation in drug analysis using electroanalytical techniques // The Open Analytical Chemistry Journal. 2014. Vol. 5, No. 1. P. 1–21. DOI: 10.2174/187406500115010001
27. *Miller J. N., Miller J. C.* Statistics and Chemometrics for Analytical Chemistry, 5th. Edition // Pearson Prentice Hall. 2005. 297 p.
28. *Silva R. B., Bulska E., Godlewska-Żyłkiewicz B., Hedrich M., Majcen N.* et al. Analytical measurements: measurement uncertainty and statistics // European Commission: Luxembourg: Publications Office of the European Union, 2012. P. 240.
29. *Grizodub A. I.* Standard validation procedures for drug quality control methods // Farmakom. 2006. No. 1/2. P. 35–44.
30. *Georgievsky V. P.* Analytical chemistry in the creation, standardization and quality control of drugs. Kharkiv: NTMT, 2011. D. 3. 1440 p.
31. *Dushna O., Dubenska L., Vojs M., Marton M., Patsay I.* et al. Highly sensitive determination of atropine in pharmaceuticals, biological fluids, and beverage on planar electrochemical cell with working boron-doped diamond electrode // Electrochimica Acta. 2022. Vol. 432. P. 141182.
DOI: https://doi.org/10.1016/j.electacta.2022.141182
32. MThech Lab: https://chem.lnu.edu.ua/mtech/mtech.htm\
33. *Marton M., Vojs M., Michniak P., Behúl M., Rehacek V.*, et al. New chemical pathway for large-area deposition of doped diamond films by linear antenna microwave plasma chemical vapor deposition // Diamond and Related Materials. 2022. No. 109111
DOI: https://doi.org/10.1016/j.diamond.2022.109111
34. *Matvieiev O., Šelešovská R., Vojs M., Marton M., Michniak P.* et al. Novel screen-printed sensor with chemically deposited boron-doped diamond electrode: preparation, characterization, and application // Biosensors. 2022. Vol. 12, No. 241.
DOI: https://doi.org/10.3390/bios12040241

ВАЛІДАЦІЯ МЕТОДИК ВИЗНАЧЕННЯ ЛІКАРСЬКИХ РЕЧОВИН НА ПРИКЛАДІ МЕТОДИКИ ВОЛЬТАМПЕРОМЕТРИЧНОГО ВИЗНАЧЕННЯ АТРОПІНУ В КРАПЛЯХ ДЛЯ ОЧЕЙ ТА РОЗЧИНАХ ДЛЯ ІН'ЄКЦІЙ

О. Душна*, Л. Дубенська

Львівський національний університет імені Івана Франка,
Кирила і Мефодія, 6, 79005 Львів, Україна
e-mail: olha.dushna@lnu.edu.ua

Валідація є важливою компонентою процедур, які повинна виконати лабораторія, перш ніж запровадити нову методику аналізу лікарських засобів. Відповідно до вимог Європейської Фармакопеї та Державної Фармакопеї України, для аналітичної методики потрібно визначати такі аналітичні характеристики: специфічність, робастність, невизначеність, лінійність, правильність, прецизійність та їхні складові.

Наведено короткий валідаційний звіт розробленої вольтамперометричної методики визначення атропіну в краплях для очей та розчинах для ін'єкцій. У роботі використано метод диференційної імпульсної вольтамперометрії і планарну плівкову комірку з робочим алмазним, легованим бором, електродом. Розроблена методика валідована за критеріями невизначеності, лінійності, правильності та прецизійності. Усі критерії витримано.

Ключові слова: валідація, ISO/IEC 17025, електрохімічні методи, вольтамперометрія, атропін.

Стаття надійшла до редколегії 25.10.2022
Прийнята до друку 17.05.2022