представництво події зростає, адже на першому саміті у 2022 році учасників і гостей було трохи більше трьохсот, а на другому – майже п'ятсот.

Як і зазвичай, кожний мав можливість самостійно обрати для себе найбільш зручний формат роботи. Ніхто не прив'язаний до стільця в залі конференцій, тому можна було слухати виступи спікерів або ж спілкуватись за чашкою кави з колегами в лаунж-зоні, відвідувати виставкові стенди чи проводити ділові переговори. Цього року саміт змінив і свою локацію – вперше він відбувся у КВЦ «Парковий», де відвідувачі оцінили комфортність роботи та якість сервісу.

Під час саміту працювала виставка обладнання та технологічних рішень у галузі тваринництва Livestock Expo 2024, на якій свої експозиції представили понад 40 компаній-постачальників продуктів, послуг та інновацій для сектору. Особливу подяку висловлюємо компаніям Vencomatic, AgriGo, Fenix Agro, RUTS та 4 Стихії, які виступили ексклюзивними партнерами заходу, та Офіційному партнеру – компанії SocTrade.

Організатори цьогорічного UKRAINIAN LIVESTOCK SUMMIT висловлюють щиру подяку за допомогу у його організації численним компаніям-партнерам, а також медіа, які інформували про його підготовку і висвітлювали саму подію.

До зустрічі на UKRAINIAN LIVESTOCK SUMMIT наступного року!

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[1]B. Zhukov, graduate student, *E-mail: o.bj0rn.c@gmail.com*
ORCID ID: 0009-0000-9860-2023
[1]A. Makarynska, Doctor of Technical Science, Associate Professor, *E-mail: allavm2015@gmail.com*
ORCID ID: 0000-0003-1879-8455, Researcher ID: C-5217-2016, Scopus ID: 57192819060
[1]T. Strakhova, Cand. of Technical Science, Associate Professor, *E-mail: straxova911@gmail.com*
ORCID ID: 0000-0002-7852-9292, ResearcherID: F-9886-2016
[2]I. Semenyuk, Cand. of Biol. Science, director, *E-mail: irina.semenyuk@sgs.com*
[1]Department of Grain and Compound Feed Technologies, Tel. no. +38048 712-40-13
Odesa National University of Technology, 112 Kanatna Str., Odesa, 65039, Ukraine
[2]Research Center for Agricultural Products and Agrotechnology, FE «SGS Ukraine», Odesa, 65000, Ukraine

USING RETROSPECTIVE DATA TO ASSESS THE UNCERTAINTY OF INDICATORS OF BIOLOGICAL SAFETY OF FOOD AND FEED RAW MATERIALS

Abstract

The results of the analysis of the advantages and disadvantages of the use of retrospective data for the implementation of the evaluation of uncertainty in the measurement of indicators of biological safety of food and feed raw materials are given. The model measurement procedure used quantitative determination of genetically modified organisms by means of polymerase chain reaction in real time 250 measurement results of certified reference material with a content of 0.1% genetically modified soybean line obtained under conditions of intra-laboratory reproducibility and 10 results of relevant inter-laboratory comparisons with the participation accredited provider. The suitability of retrospective data was assessed by the Shapiro-Wilk criterion and statistical controllability analysis using standardized score charts and modified Shewhart charts, uncertainty by 4 methods described in general and branch international guidelines and regulations. The results of using a combination of modified Shewhart maps demonstrate the acceptability of this approach for establishing the suitability of retrospective measurement results as input data for uncertainty assessment. By integrating additional "fitness limits" to a set of standardized control limits, this analysis acquires the ability to simultaneously establish not only stability, through the interpretation of geometric patterns, but also compliance with specific criteria. Using this approach, objective evidence of the suitability of retrospective data for estimating measurement uncertainty was obtained. The distribution of retrospective data obtained under conditions of intralaboratory reproducibility is assumed to be normal. As a result of the assessment of statistical controllability and compliance with the established criteria, objective evidence of the suit-

ability of the data for uncertainty assessment was obtained. Analysis of uncertainty intervals of subgroups with the volume of 25 measurements, using 4 assessment methods, demonstrated significant variability in the results of measuring biological safety indicators. The uncertainty interval could narrow or widen by almost 100% of the value within fifty measurements. Median values obtained using different uncertainty estimation methods were 2.69%, 22.69%, 39.23, and 43.08%. This variability is due to the limited coverage of sources of uncertainty, which is inherent in each of the analyzed methods. The most optimal method is the evaluation based on the routine measurement of the certified reference material. Retrospective results of routine measurement of certified reference material and interlaboratory comparisons are an effective source of input data for assessing the uncertainty of the results of measuring biological safety indicators of agricultural raw materials. There is a need for further research to establish an assessment model that will provide an opportunity to take into account the optimal number of influential sources of uncertainty that are inherent in biological systems and cannot be simultaneously taken into account using existing approaches offered by standard assessment methods.

Key words: control, quality, safety, statistics, uncertainty, measurement.

Introduction

Assessment of compliance of agricultural products with established safety criteria is carried out in accordance with analytical results provided by the laboratory. Mutual recognition of such results, which is based on accreditation by bodies that have an international MRA (The Mutual Recognition Arrangement), requires the presence of a procedure for assessing the uncertainty of measurements carried out by the laboratory, at least within the scope of accreditation. This requirement is of significant importance because, in addition to the established wide list of indicators (Directive 2002/32/EC of 7 May 2002. OJ L 140, 30.5.2002; Commission regulation EC № 1881/2006 of 19 December 2006. OJ L 364, 20.12.2006), the concept of safety requires a risk-oriented approach to decision-making, which should be based on taking into account the uncertainty of the results of measuring quantitative indicators ILAC G17:01.

Thus, knowledge of the uncertainty interval is of crucial importance for all stakeholders who interpret the results of the analysis of safety factors, as it forms the conditions for finding a reasonable balance between the error of the first and second kind ASME B89.7.3.1 – 2001 R2019.

Due to the variability inherent in almost all components of biological systems, an effective Type B uncertainty assessment, with a detailed calculation of the contribution of each individual source, may be limited in applicability or unable to provide the required level of detail. In such a case, a direct determination of the combined uncertainty, taking into account the influence of all or most of the type A sources, may be most appropriate (Ellison et al., 2012). Nevertheless, establishing the actual uncertainty interval of the current method, even with the help of this approach, is not always economically feasible and able to provide optimal coverage of influential sources, since their detailed evaluation with the inclusion of a sufficient sample volume and duplication of measurements can lead to significant material costs.

The use of retrospective data can have a positive impact on both the quality and the economic component of the type A uncertainty assessment procedure. Such data can include a database of routine measurement of a certified reference material (CRM) or an intra-laboratory test of a blind sample, a chain of inter-laboratory comparisons with the participation of an accredited provider (ICAP), etc. However, in the case of using such data, it is necessary to substantiate their suitability, taking into account changes that could affect the characteristics of the method during the time from the moment of receipt (Ellison et al., 2012).

The purpose of this study was to evaluate the suitability of modified control charts with an integrated additional set of "limits of agreement" for making a conclusion about the possibility of using the data to estimate the uncertainty of measurement, and to analyze the suitability of the type A approach to uncertainty estimation, using retrospective data.

To achieve the goal, the following tasks are set:

1. To evaluate the suitability and features of the application of modified control charts to establish the statistical controllability of the process and analyze the compliance of the results of retrospective measurements of biological analytes with the requirements for accuracy and stability, within the framework of the formation of an evidence base that the results of these measurements can be objectively applied taking into account changes, which could affect the process over time.

2. Conduct an analysis of existing approaches to uncertainty assessment, including:

-estimation of the combined standard uncertainty using the standard deviation of the sample, calculated relative to the arithmetic mean of a series of measurements performed under reproducibility conditions;

-estimation of the combined standard uncertainty using the standard deviation of the sample, calculated relative to the reference value, taking into account the bias;

-assessment of the combined standard uncertainty using the standard deviation of the general population, calculated by an accredited provider based on the results of an interlaboratory comparison;

-assessment of the combined standard uncertainty using the standard deviation of the general population, calculated by an accredited provider based on the results of an interlaboratory comparison, taking into account the laboratory bias;

3. To compare the results obtained using four approaches to the assessment of measurement uncertainty, taking into account the requirements for the use of extended uncertainty in decision-making regarding the safety of food and fodder agricultural raw materials.

4. To analyze the suitability of the uncertainty assessment using a retrospective analysis of the sequence of results of routine measurement of SRM, the statistical controllability of which is confirmed by modified control charts, and the chain of results of interlaboratory comparison with the participation of an accredited provider.

Materials and methods

Model measurement procedure. A sample consisting of the results of 250 consecutive routine measurements of the concentration of genetically modified soybean line MON 40-3-2 in SRM with an assigned con-

centration of 0.1%, which was taken as a reference value, was used as a model for practical testing of the researched concept. These measurements were carried out in conditions of intra-laboratory reproducibility in the period from 04.02.2019 to 21.11.2022 at the testing center of the SP "SZHS Ukraine".

The method of determining the content of GMOs was used as a model due to the fact that this procedure contains a significant number of operational stages, which is carried out by different operators, requires the use of SRM during routine measurement, has relatively low limits for the qualitative and quantitative determination of the analyte, and returns a result with accuracy to the thousandth.

Research materials. The research material was the results of measurements of four batches of CPM line of genetically modified soybean MON 40-3-2 produced by ERM (European Reference Material), catalog number ERM-BF410dp. Compliance with the requirements of ISO 17034 is confirmed by quality certificates under numbers 0516 dated 03/13/2019, 0833 dated 10/01/2019, 0943 dated 10/10/2022 0944 dated 10/10/2022 ISO 17034:2016. The certified attributed concentration value of the reference material was 10.0 g/kg, which was equal to 0.1% of the GM content of the material, with an expanded uncertainty of each batch of ±0.6 g/kg.

In addition, the results of the participation of the testing center of the SP "SZHS Ukraine" in ten rounds of interlaboratory comparisons with the participation of an accredited provider, by the method of quantitative determination of the content of genetically modified soybeans by the polymerase chain reaction method in real time, with the participation of the provider "Bipea" ISO 9001 accreditation certificate No. 9912427 were used , ISO 17043 accreditation certificate #1-1495 for the period from 2014 to 2019 inclusive.

Identification of sources of uncertainty. The identification of sources of uncertainty that had or could have an impact on the measurement result was performed using the method of root cause analysis with graphical display using the method of Ishikawa diagrams (Ishikawa, 1985) and according to ISO 31000:2018 and ISO 31010:2019 (Cause and Effect Diagram).

Evaluation of the statistical controllability of the process. Checking the normality of the distribution of the research sample was carried out according to the Shapiro-Wilk test (Jitendar Vij, 2011; Buja et al., 2009), according to the formula regulated by ISO 5479:1997:

$$W = \frac{[\sum_{i=1}^{m} a_{n-i+1}(X_{n-i+1} - y_i)]^2}{\sum(X_i - X_{ave})^2}, \quad (1)$$

where W – is the Shapiro-Wilk criterion;

X_i – is the individual value of the sample, which was previously sorted according to the non-ascending principle;

Xave – is the arithmetic mean of the sample;

a_i – table coefficient;

m – is a coefficient equal to (n-1)/2 for samples consisting of an even number of measurements and n/2 for samples with an odd number of measurements.

The assessment of the state of statistical controllability of the measurement method was carried out using the analysis of the standardized score (Z-score) and quantitative Shewhart control charts (Novikov et al., 2002) for the distribution of ranges without a given standard value and the distribution of individual values with a given standard value, according to ISO 7870-1:2019 and ISO 7870-2:2023.

The indicators of the standardized assessment were calculated using the formula:

$$Z = \frac{X_i - X_0}{Sd}, \quad (2)$$

where Z – is a standardized score;

X0 – reference value;

Xi – individual sample value;

Sd is the standard deviation of the sample.

As a reference value of X0, the indicators of the average arithmetic sample and the value of the certified reference material for n=250 were consistently used. The standard deviation of the sample in the calculation corresponded to the volume of the sample to be analyzed.

The 3σ indicator was chosen as the criterion of conformity of the indicators of the standardized assessment, which corresponds to taking into account 99.7% of the values of the measured value according to the Gauss-Laplace distribution.

To establish statistical controllability using quantitative Shewhart maps, the total sample with the volume of 250 measurements was divided into 5 subsamples with the volume of 25 measurements. As the central line of the map of individual values with a given control value, the reference value of the SRM was used. The control limits were calculated according to the formulas regulated by ISO 7870-2:2023:

$$\text{UCL} = X_0 + 3 \times Std, \quad (3)$$

$$\text{LCL} = X_0 - 3 \times Std, \quad (4)$$

where UCL – is the upper control limit;

LCL – lower control limit;

X_0 – is the attributed value of the certified reference material;

Std – standard deviation of the sample;

The center line of the swing map without a given standard value was the average value of the difference between the maximum and minimum value of the measurement. The control limits were formed according to the formulas regulated by ISO 7870-2:2023:

$$\text{UCL} = D_4 \times \Delta_{ave}, \quad (5)$$

$$\text{LCL} = D_3 \times \Delta_{ave}, \quad (6)$$

where Δave – is the average range of the sample;

D_3, D_4 – are constant table values.

Violation of the calculated upper or lower control limits is accepted as the compliance criterion for evaluating statistical controllability using quantitative Shewhart maps, using the given parameter X0, determined by the certified reference material. The criterion of stability, which was used both for the map with the specified and for the map without the specified parameter, was the interpretation of the patterns of the distribution of values according to the rules of Westgaard (Westgard et al., 1977; Westgard et al., 1981; Westgard et al., 1990) and the rules given in the ISO 7870-2:2023 standard.

Admissible values based on an indicator equal

to 20% of the deviation of the measurement result from the reference value were accepted as additional evaluation criteria when using quantitative Shewhart maps. Thus, for the swing control chart, 0.2 points was taken as the maximum acceptable value, which formed the suitability interval from 0 to 0.2 points. For control charts of individual values, the minimum and maximum acceptable value formed a suitability interval from 0.8 to 1.2 points. These indicators are integrated into the control charts as an additional set of "compliance limits".

Evaluation of measurement uncertainty. Uncertainty assessment was carried out according to type A, with the help of mathematical processing of the results obtained by multiple measurements of SRM in conditions of intra-laboratory reproducibility and the results of participation in inter-laboratory comparisons according to BIPM JCGM 200 2012 - International vocabulary of metrology and ISO/IEC Guide 99:2007 International vocabulary of metrology.

The starting concept of the calculation of the combined standard measurement uncertainty (Combined standard measurement uncertainty, uc), i.e., one that includes all or most of the important sources, is the square root of the sum of the squares of the standard uncertainty of precision and the standard uncertainty of bias.

The calculation of the standard uncertainty, based on the arithmetic mean of repeated measurements, was carried out according to the formula regulated by EURACHEM/CITAC Guide CG 4 (Ellison et al., 2012; Lavs′ka,2023; Yeremenko, 2013):

$$u_{cAve} = \frac{\sqrt{\frac{\sum(X_i - X_{Ave})^2}{n(n-1)}} \times 100}{X_{ave}}, \quad (7)$$

where ucAve – is the relative combined standard uncertainty of the mean value;

X_i – measurement result;

X_{ave} – is the arithmetic mean of measurement results,

n – sample volume.

This calculation is relevant for methods that do not have the ability to rely on the value of the certified reference material, with the corresponding calculation of the bias contribution to the combined uncertainty. Thus, for this category of methods, the standard uncertainty of the mean value is taken as the combined uncertainty. For the analysis of this uncertainty assessment method, the experimental sample was taken regardless of the reference value of the SRM and relied on the arithmetic mean indicator.

The combined uncertainty, based on the dispersion relative to the reference value, taking into account the bias, was calculated according to the formula regulated by FAO CALC/GL 59-2006 and SANTE 11312/2021:

$$u_{cRL} = \sqrt{\left(\frac{Std_X * 100}{X_{ave}}\right)^2 + \left(\sqrt{\left(Ave_\Delta, \% \left(\frac{\sum\left(\frac{\Delta_i \times 100}{X_i}\right)}{n}\right)\right)^2 + \left(Std_\Delta, \% \left(\frac{\Delta_i \times 100}{X_i}\right)\right)^2}\right)^2}, \quad (8)$$

where u_{cRL} – is the relative combined standard uncertainty based on the measurement of the certified reference material;

Std_X – standard deviation of the measurement results of the certified reference material;

X_{ave} – is the average value of the certified reference material;

Ave_Δ – is the arithmetic mean of the differences between the reference value and the measurement result, expressed in relative values from the measurement result;

Std_Δ – is the standard deviation of the differences between the reference value and the measurement result, expressed in relative values from the measurement result.

Two approaches were used to estimate the uncertainty based on the results of the interlaboratory comparison.

In the first approach, as an indicator of combined uncertainty, the indicator of the standard deviation of the general population, which is determined by the provider, was taken. In this way, the category of methods that cannot rely on the value of the certified reference material was modeled, with the corresponding calculation of the bias contribution to the combined uncertainty.

The standard uncertainty, which is not based on the results of interlaboratory comparisons, including the bias, was calculated according to the formula regulated by FAO CALC/GL 59-2006 and SANTE 11312/2021:

$$u_{cIC} = \sqrt{\left(\frac{Std_{IC} * 100}{X_{IC}}\right)^2 + \left(\frac{\Delta_i \times 100}{X_i}\right)^2}, \quad (9)$$

where u_{cIC} – relative combined standard uncertainty based on results of interlaboratory comparison;

Std_{IC} – standard deviation of interlaboratory comparison;

X_{IC} – reference value of interlaboratory comparison;

Δ_i – the difference between the reference value and the result obtained by the laboratory when participating in the interlaboratory comparison;

X_i – is the measurement value obtained by the laboratory in an interlaboratory comparison.

In this formula, to exclude the influence of the bias of other laboratories, the determination of ubias was performed using △ only the laboratory on the basis of which the results of the measurements under investigation were obtained. Therefore, Std△ was set to 0, and the standard bias uncertainty was taken as the relative difference between the reference value and the target laboratory's measurement result. The extended measurement uncertainty was calculated according to the formula regulated by BIPM JCGM 101:2008 and ISO/IEC Guide 98-3:2008/Suppl 2:2011:

$$U = u_c \times k, \quad (10)$$

where u_c – is the combined standard measurement uncertainty;

U – expanded measurement uncertainty;

k – is the coverage factor.

The coverage factor (Coverage factor, k) is taken equal to 2, which corresponds to p=0.05 and provides consideration of approximately 95% of the distribution of values near the normalized average.

Results and discussion

Identification of sources of uncertainty. Based on the identification of sources of uncertainty that affect or may affect the result of measuring the content of genetically modified soybeans using polymerase chain reaction in real time, six basic categories of sources were identified. A graphical representation of the results of source identification is shown in Figure 1.

To analyze the influence of the human factor, the sample preparation process was carried out by six operators, the DNA extraction process by six operators, the polymerase chain reaction process by five operators, on the basis of which the measurement results were registered. In total, eleven laboratory personnel were able to or influence the test results. During the retrospective data collection, the areas of influence of some operators changed regularly, while other operators were assigned to perform a specific operation. According to the requirements of the analytical method, each operator performed only one operation during the working day.

In retrospect, the sample preparation procedure was performed by six operators, the DNA extraction procedure by six operators, and the polymerase chain reaction procedure by five operators, according to which the measurement results were recorded. The total number of operators who could or did influence the test result, which was taken into account in the retrospective analysis, was eleven laboratory employees. In the process of accumulating retrospective data, regular changes were made in the sphere of influence of some operators, while other operators were fixed on the performance of a certain operation. According to the requirements of the analytical method, each operator performed only one operation during the work shift.

Taking into account the variability factor over time, data accumulation was carried out during four years of SRM measurement and six years of participation in interlaboratory comparisons.

Control of the environmental impact was carried out by means of daily registration and comparison of the compliance of indicators with the criteria stipulated in the technical documentation of the equipment, consumables and regulatory documentation: ISO 21571:2005/Amd 1:2013 Foodstuffs; ISO 21569:2005/Amd 1:2013 Foodstuffs; ДСН 3.3.6.042-99; ISO/IEC 17025:2017.

The effect of the sample on the measurement result was evaluated using four batches of SRM and ten samples provided by an accredited interlaboratory comparison provider;

Taking into account the impact of the equipment, alternative equipment that performs the same function was used. This list included changes between three amplifiers, four thermostats, three centrifuges, three scales, and ten variable volume dispensers. Measuring equipment was subject to regular annual calibration and assessment of statistical controllability in the intercalibration interval, in accordance with current regulatory requirements ISO/IEC 17025:2017.

Consumables included commercial extraction and amplification kits, replaceable single-use dispenser tips, single-use microtubes, and amplification plates, which were renewed as they were used during the data collection period.

The measurement procedure is accepted as a non-alternative factor that has not undergone modifications in accordance with the annual update of the basic regulatory documents.

Evaluation of the statistical controllability of the process. To obtain the possibility of stepwise control of the data, followed by evaluation using Shewhart maps, descriptive statistics analysis was performed for the total sample and subsamples with n=25. Descriptive statistics obtained during the analysis of retrospective data of SPM measurement in conditions of intralaboratory reproducibility are shown in Table 1.

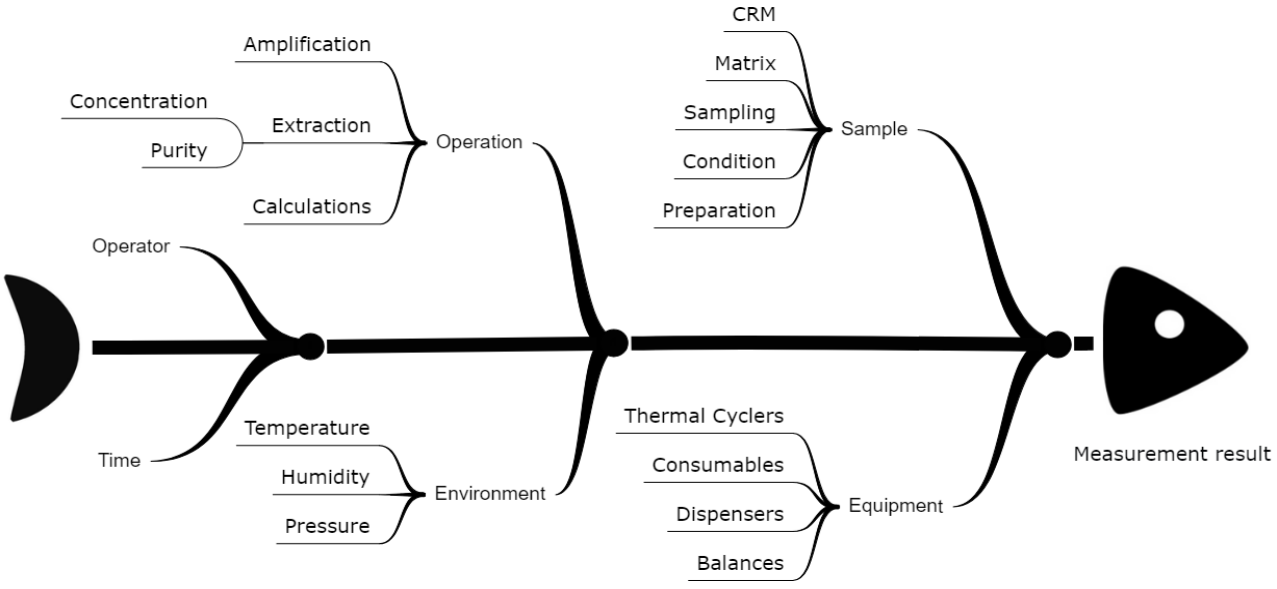


Fig. 1. Identification of sources of uncertainty using the Ishikawa diagram

Table 1 – Descriptive statistics of the sample of SRM measurement results

Sample	Statistics					
	Minimum	Maximum	Range	Average	Mode	Median
1-250	0,081	0,120	0,039	0,102	0,102	0,102
1-25	0,088	0,116	0,028	0,103	0,102	0,103
26-50	0,089	0,106	0,017	0,099	0,100	0,100
51-75	0,082	0,111	0,029	0,101	0,102	0,102
76-100	0,081	0,118	0,037	0,100	0,109	0,100
101-125	0,093	0,118	0,025	0,105	0,106	0,106
126-150	0,081	0,117	0,036	0,099	0,102	0,102
151-175	0.087	0,116	0,029	0,106	0,111	0,106
176-200	0.090	0,119	0,029	0,105	0,115	0,105
201-225	0,093	0,116	0,023	0,103	0,107	0,102
226-250	0,084	0,120	0,036	0,101	0,101	0,101

Table 2 – Results of testing the normality of the sample distribution using the Shapiro-Wilk test

W	Subgroup				
	1-50	51-100	101-150	151-200	201-250
Experimental	0,9663	0,9214	0,9328	0,7539	0,9706
Critical (p 0,05, n 50)	0,9470				
Critical (p 0,01, n 50)	0,9300				
Normality	+	-	-	-	+

Table 3 – Descriptive statistics of two approaches to calculating the standardized score

Statistical indicator	Z from the reference value	Z from the mean value
Minimum value	-2,64	-2,37
Maximum value	2,22	2,49
Arithmetic mean	0,00	0,27
Median	-0,02	0,25
Fashion	-0,02	0,25
Swing	4,86	4,86

To assess normality using the Shapiro-Wilk W test, the total sample was divided into five subgroups of 50 measurements. The results of the evaluation are shown in Table 2.

According to the obtained data, group 1-50 and 201-250 showed a normal distribution of data at p 0.05. Group 101-150 demonstrated normality at p 0.01. For groups 51-100 and 151-200, not enough data were obtained to make a conclusion about the normality of the distribution. Accordingly, taking into account EA-4/02 M:2022, it was concluded that the data of the subgroups and the total sample were removed from the normal distribution. Accordingly, the data are suitable for further analysis.

Analysis of standardized assessment. According to the results of the assessment of the stability of the measurement process using a standardized assessment, no contradictory values were obtained, which would indicate the influence of special factors on the results. Violations of the established requirements for compliance were not detected. Descriptive statistics of the standardized score are shown in Table 3.

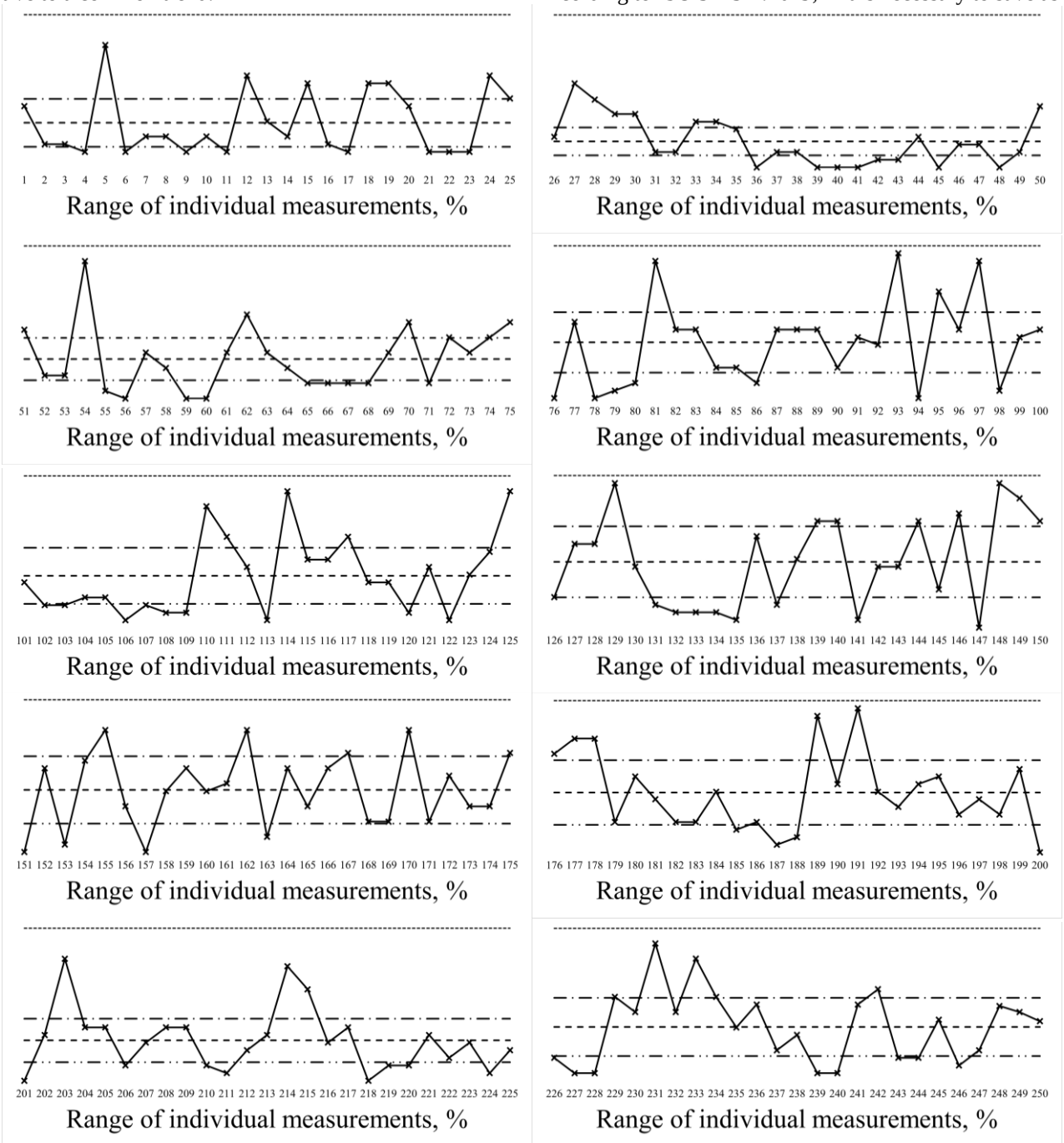
It should be noted that the standardized assessment, which is based on the reference value, forms more accurate data. Z indicators obtained from the reference value have a more centered position compared to the standardized estimate from the arithmetic mean. According to descriptive statistics, despite the fact that both approaches account for the same measure of swing, the location relative to the centerline, the Z-score relative to the mean, shows a bias of about 0.3 standard deviations toward overestimation, according to the study sample. This can be a significant bias in the case of methods of research of biological indicators that have a high sensitivity to external factors and for samples with a small volume, in which the mathematical expectation can differ significantly from the real state of the indicator.

Analysis of Shewhart's quantitative maps. According to the result of the evaluation of the process using quantitative Shewhart maps, for the analysis of the dynamics of swings, no signs of exit from the state of controllability were found. The set maximum permissible

value was not violated. A graphical representation of the assessment is shown in Figure 2.

As can be seen from the displayed results, the map containing the data of measurement spans from 26 to 50 shows relatively shifted control limits. This is because the average range of measurements for this sample was 0.003 percent, which is the lowest value among all ten subgroups, while the median value was 0.007 percent. However, this displacement does not exclude the possibility of using these limits as a geometric reference for evaluating the stability of the results. This case emphasizes the need to study not only the largest possible sample, but also the joint analysis of grouped data relative to a common trend.

More important for this subgroup is the comparable decrease in the average range of the measurement results, since taking into account the results of this subgroup can affect the result of the uncertainty assessment, leading to a narrowing of the final uncertainty interval. Due to the fact that the factor that led to such a decrease in the average span had an impact only within the subgroup 26-50, this set of measurements can be perceived as a drop with the subsequent exclusion of its indicators from the calculation. Alternatively, it may be recommended to analyze this subgroup for outliers in the middle of the data, for example by calculating the Cochrane test or the Grubbs test (Yeremenko et al., 2013) and according to ISO 5725-2:2019, if it is necessary to save as



R - range, UCL - upper control limit, CL - central line,
LCL - lower control limit, ULC - upper limit of compliance

Fig. 2. Graphical representation of the evaluation of the statistical controllability of the process using control maps of swings

much data as possible. In addition, it should be emphasized the difficulties that arise when using a single list of patterns to make a conclusion about the exit of the process from the state of stability. To carry out an effective analysis, it is necessary to focus on the characteristics of the process that are formed in certain conditions of its implementation, which also demonstrates the prospects of processing retrospective data.

According to the result of evaluating the process using Shuhart's quantitative maps, for the analysis of the dynamics of individual measurement results, no signs of exit from the state of controllability were found. The minimum and maximum permissible values of the individual measurement were set and were not violated. A graphical representation of the assessment is shown in Figure 3.

As can be seen in the graphs, maps based on a standardized value, such as SRM, do not form sufficiently rigid control limits to ensure compliance with established requirements. In this regard, additional limits are integrated into the map, which form the correspondence interval based on the minimum and maximum permissible values. As you can see, this interval has an area almost 60% narrower than the interval of control limits.

According to the conducted study, the suitability of modified statistical maps to meet the requirements for the formation of objective evidence of the suitability of retrospective data for the assessment of measurement uncertainty has been demonstrated. On the basis of the conducted assessment, the statistical controllability of the analytical method in the specified time period and the

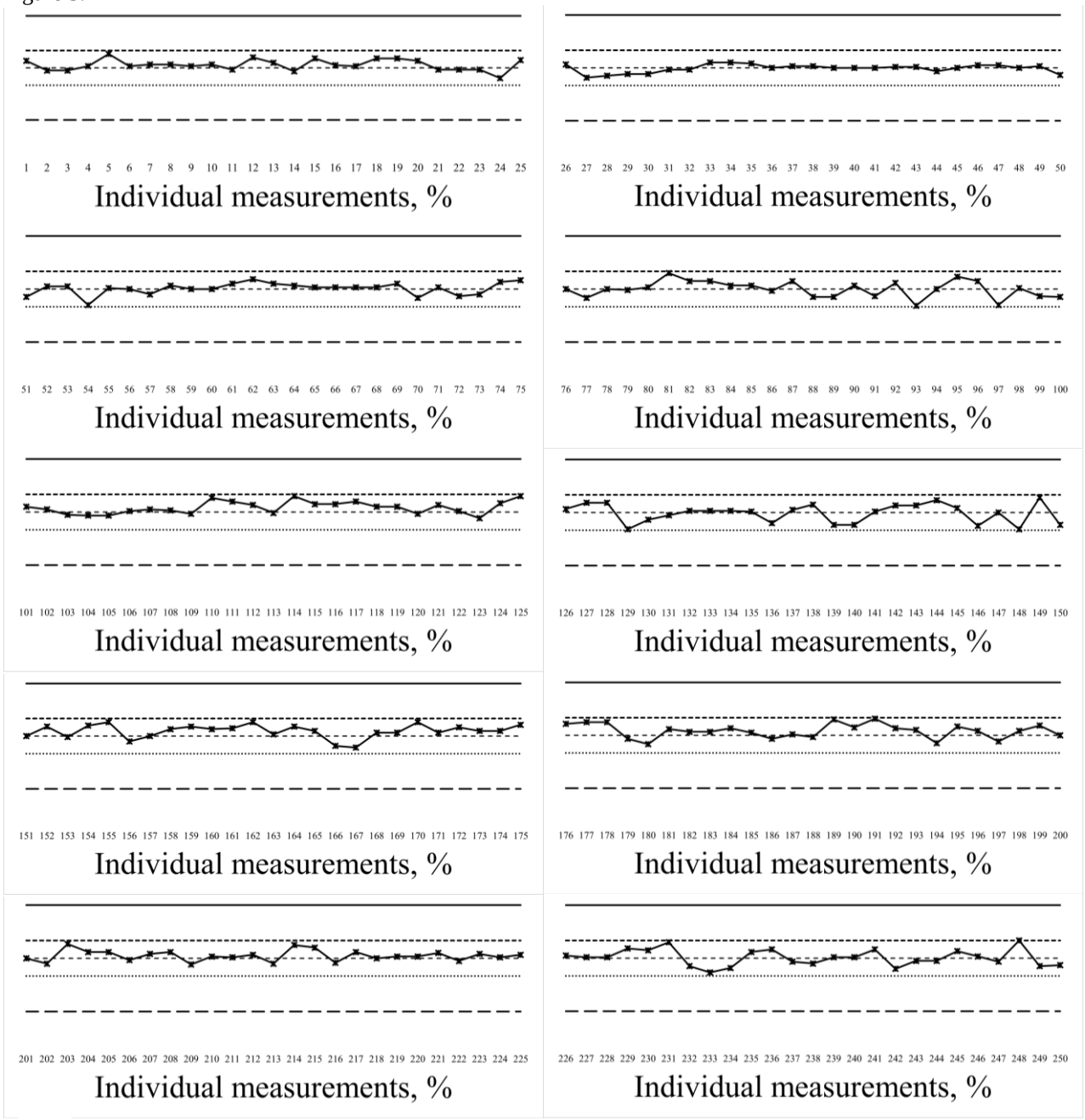


Fig. 3. Graphic representation of the assessment of the statistical controllability of the process based on the results of individual measurements

Table 4 – Results of uncertainty assessment based on the arithmetic mean of samples

Sample	u	U	URel, %
1-250	0,0005	0,001	0,993
1-25	0,0013	0,003	2,48
26-50	0,0009	0,002	1,877
51-75	0,0014	0,003	2,687
76-100	0,0019	0,004	3,752
101-125	0,0014	0,003	2,68
126-150	0,0021	0,004	4,305
151-175	0,0015	0,003	2,925
176-200	0,0016	0,003	3,063
201-225	0,0012	0,002	2,37
226-250	0,0019	0,004	3,696

Table 5 – Results of uncertainty assessment based on reference value of SRM

Sample	ubias, %	uprecision, %	uc, %	URel, %
1-250	8,1894	7,8481	11,343	22,686
1-25	6,7291	6,2729	9,199	18,399
26-50	4,9266	4,5656	6,717	13,434
51-75	7,1615	6,6135	9,748	19,496
76-100	9,7829	9,169	13,408	26,816
101-125	7,6478	6,8855	10,291	20,581
126-150	11,5301	10,4492	15,56	31,121
151-175	8,8437	7,5634	11,637	23,274
176-200	8,5181	7,87	11,597	23,194
201-225	6,1959	5,9696	8,604	17,208
226-250	9,0563	9,1052	12,842	25,684

Table 6 – Results of uncertainty assessment based on interlaboratory comparisons with the participation of an accredited provider

Year of implementation	URel від Pt, %	URel від Pt +bias, %
2014	50,000	50,000
2015	40,000	40,000
2016	31,579	31,579
2017	40,000	56,569
2018	46,154	46,154
2019	35,294	37,203
2020	46,154	55,470
2021	30,769	68,802
2021	31,818	39,101
2022	38,462	39,223

suitability of retrospective data for uncertainty assessment were proven.

Evaluation of measurement uncertainty. The results of the uncertainty assessment based on the calculation of the dispersion of the results of independent measurements carried out under conditions of intra-laboratory reproducibility relative to their arithmetic mean are shown in Table 4.

The results of the uncertainty assessment, based on the calculation of the dispersion of the results of independent measurements carried out under conditions of intra-laboratory reproducibility relative to the reference value of the certified reference material, taking into account the bias, are shown in Table 5.

To examine the potential reduction in uncertainty estimation that was likely to occur under the conditions of using data from 26 to 50, additional calculations were performed excluding them from the total sample. According to the results, the uncertainty based on the arithmetic mean of the sample excluding the 26-50 subgroup was equal to 1.441% compared to 0.993% obtained in the analysis of the full sample. The uncertainty based on the reference value excluding the 26-50 subgroup was 23.392% compared to 22.924% obtained when analyzing the full sample.

In accordance with this, it should be noted that the emission has a much greater influence on the uncertainty estimate, which is based on the calculation of the dispersion of the results of independent measurements carried out in conditions of intra-laboratory reproducibility relative to their arithmetic mean. The effect of the 26-50 subgroup resulted in a 69 percent change in the uncertainty range compared to the uncertainty calculated from the full data sample. Because this approach relies on mathematical expectation, it shows much greater sensitivity to sample size, distribution of values, and outliers.

The effect of the 26-50 subgroup on the uncertainty calculation based on the reference value resulted in a range change of only 2% compared to the uncertainty calculated from the full data sample. In the example under analysis, this is interpreted as a minor deviation.

Further comparisons and analyzes were performed with indicators calculated using the full sample.

The results of the uncertainty assessment based on the results of interlaboratory comparisons are shown in Table 6.

According to the obtained data, a comparative graph of the dynamics of uncertainty, which was obtained using four different approaches, was formed.

This graph is shown in Figure 4.

As can be seen from the given diagram, the measurement uncertainty has significant variability over time. This is due to the changes that inevitably occur among the factors that exert or can exert an influence on the measurement result. According to the studied data, the uncertainty interval within fifty measurements made during one month can narrow or expand by almost 100% of its value and by more than two times during the studied period, despite the proven stability and compliance of the process.

According to this, despite the lack of a direct requirement to update information on uncertainty, which is already established for the test method during the corre-

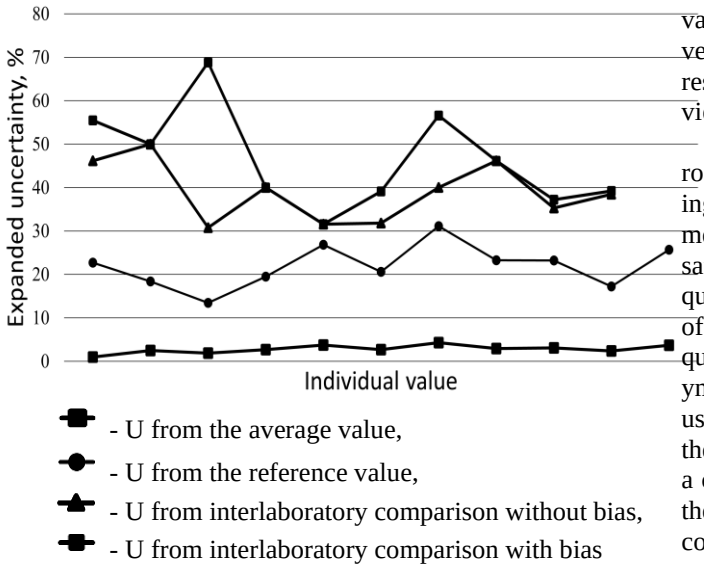


Fig. 4. Dynamics of indicators of relative expanded uncertainty obtained by four assessment approaches

sponding verification study ISO/IEC 17025:2017, there is a need to periodically reevaluate its interval. In connection with the constant updating and strengthening of requirements for the safety of food and fodder agricultural raw materials, the influence of not only qualitative assessment of the uncertainty of the results of relevant measurements, but also taking into account the variability of this indicator, to ensure national and international compliance of products, is increasing.

In accordance with this necessity, the question of the most appropriate method of controlling the dynamics of uncertainty arises. The main requirements for the optimal method are to ensure the necessary level of evaluation quality, which can satisfy the interested parties who interpret the measurement result, and the economic feasibility of the evaluation procedure.

Given the results of the analysis of the dynamics of the uncertainty assessment results, it is obvious that the periodic repetition of the verification experiment in order to establish the uncertainty is insufficient. This is because the verification procedure provides only a point-by-point understanding of the uncertainty interval that is relevant to the state of the associated sources, which have significant variability, especially in relation to testing biological analytes. Even considering that not all uncertainty factors have the same degree of influence on the measurement result (Ellison et al., 2012), sources such as equipment, operators, and time form a significant contribution to uncertainty and are characterized by significant variability over time. In addition, periodic updating of the verification is associated with significant expenditure of resources, which is not always justified from the point of view of the result.

An alternative to experimental updating is a retrospective analysis, in particular of the results of measuring certified reference material. A large number of test methods aimed at establishing indicators of biological safety of food and fodder agricultural raw materials require the use of certified reference material in the process of routine research. Examples of these methods can be quantitative molecular genetic methods based on the polymerase chain reaction, enzyme immunoassay methods using measurements of optical density, measurement of the content of mycotoxins, etc. Thus, using the results of a chain of routine measurements of the certified value of the reference material as a reference value for uncertainty control is an approach with sufficient justification.

However, for some methods of quantitative analysis of biological analytes, there are no control samples. An example of this may be methods of microbiological research based on counting the number of colonies, membrane filtration or the most probable number. Obtaining a reference value for such methods is a difficult task, which is traditionally solved by using the mathematical expectation described by the arithmetic mean of the sample.

Another alternative is to use the standard deviation of interlaboratory comparisons. However, this approach cannot take into account a large number of sources, and the uncertainty estimate comes from the contributions of the laboratories of the comparison participants, limiting the description of specific laboratory conditions. The joint influence of these factors can form an inflated uncertainty interval.

A comparison of the relative expanded uncertainties obtained by these approaches is shown in Table 7.

According to the obtained data, the median level of uncertainty obtained using the evaluation from the reference value is more than thirteen times higher than the calculations based on the arithmetic mean of the sample. In turn, this interval is less than two times lower than the uncertainty estimated by interlaboratory comparison.

The approach to uncertainty assessment using the results of interlaboratory comparison without taking into account the bias of the laboratory and taking it into account, shows a difference in median values of 1.4% of the result.

Table 7 – Comparison of values of relative expanded uncertainties obtained by different approaches

Evaluation method	Indicators of extended uncertainty, %			
	Minimum	Median	Maximum	Range
From the average value	0,99	2,69	4,31	2,43 (56,38%)
From the reference value	13,43	22,69	31,12	17,86 (56,83%)
From interlaboratory comparison without bias	30,77	39,23	50,00	19,23 (38,46%)
From interlaboratory comparison with bias	31,58	43,08	68,80	37,22 (54,10%)

Conclusions

The combination of standardized evaluation maps and Shewhart control maps with the addition of lines of maximum and minimum acceptable values to the set of control limits have high prospects in terms of providing objective evidence of the suitability of retrospective data for assessing measurement uncertainty. The use of modified maps makes it possible to reasonably assert the statistical controllability, stability and compliance of the measurement procedure in a specific period of time, based on the analysis of variability and average values of measurements.

Analysis of the dynamics of uncertainty estimates during six years of interlaboratory comparisons and four years of intralaboratory routine measurement of certified reference material demonstrate significant fluctuations in the measurement uncertainty indicator, which underwent changes of almost 100% of the estimated value during the experimental period. This variability demonstrates the need for periodic updating of measurement uncertainty assessment to support the ability to make informed decisions regarding biological safety indicators of food and feed agricultural raw materials.

Despite the ability to develop a qualitative design that takes into account a large number of sources, an estimation approach based on the arithmetic mean of the sample returns narrow uncertainty intervals that cannot adequately account for bias, and at the same time is highly dependent on the size of the sample and the presence of outliers among measurement results. In addition, it should be emphasized that, according to the obtained data, the arithmetic mean indicator is not always the optimal measure of the central tendency in the conditions of methods of analysis of biological indicators. Therefore, this assessment method should not be preferred when an approach based on a higher quality evidence base can be used, including the use of certified reference material and interlaboratory comparisons with the participation of an accredited provider.

The use of a sample of measurement results of certified reference material enables a balanced assessment of uncertainty, taking into account the contributions of precision and bias components. Along with this, this method, in the case of a positive assessment of the statistical controllability of the process, has a significant evidential basis. However, it should be taken into account that this approach may not be able to take into account such a critical source of uncertainty as the sample preparation procedure, which often includes such steps as sample homogenization, analyte release or extraction, uniform distribution of the measurement object, and other influencing factors.

An approach based on the results of interlaboratory comparisons with the participation of an accredited provider provides a significant evidence base, but takes into account the measurement conditions of all laboratories participating in the comparison. This forms a distance from the peculiarities of the implementation of the analytical procedure in the conditions of a certain laboratory according to its individual influences of sources of uncertainty. In this regard, the obtained intervals may significantly exceed the practical uncertainty of this specific laboratory.

According to the obtained data, significant advantages of using retrospective data in comparison with the revalidation experiment have been demonstrated, however, there is a need for further research in order to combine in one design the uncertainty assessment of the largest number of sources and to provide the most reasonable initial uncertainty interval for measuring the biological safety indicators of food and feed raw materials.

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[1]Б. Жуков, аспірант, *E-mail: o.bj0rn.c@gmail.com*
[1]А. Макаринська, д-р техн. наук, доцент, *E-mail: allavm2015@gmail.com*
[1]Т. Страхова, канд. техн. наук, доцент, *E-mail: straxova911@gmail.com*
[2]Семенюк І.В., канд. біол. наук, директор, *E-mail: irina.semenyuk@sgs.com*
[1]Кафедра технології зерна і комбікормів, тел. +38048 7124013
Одеський національний технологічний університет, вул. Канатна, 112, м. Одеса, 65039, Україна
[2]Науково-дослідний Центр Агро-продукції та Агро-технології, ІП «СЖС Україна». Одеса, Україна

ВИКОРИСТАННЯ РЕТРОСПЕКТИВНИХ ДАНИХ ДЛЯ ОЦІНЮВАННЯ НЕВИЗНАЧЕНОСТІ ВИМІРЮВАНЬ ПОКАЗНИКІВ БІОЛОГІЧНОЇ БЕЗПЕКИ ХАРЧОВОЇ ТА КОРМОВОЇ СИРОВИНИ

Анотація

Наведено результати аналізу переваг та недоліків використання ретроспективних даних для реалізації оцінювання невизначеності вимірювання показників біологічної безпечності харчової та кормової сировини. Модельною процедурою вимірювання використано кількісне визначення генетично модифікованих організмів за допомогою полімеразної ланцюгової реакції у режимі реального часу 250 результатів вимірювання сертифікованого референсного матеріалу з вмістом 0,1% лінії генетично модифікованої сої, що отримано в умовах внутрішньо лабораторної відтворюваності та 10 результатів відповідних міжлабораторних порівнянь з участю акредитованого провайдера. Придатність ретроспективних даних оцінювали за критерієм Шапіро-Уілка та аналізу статистичної контрольованості з використанням карт стандартизованої оцінки і модифікованих карт Шухарта, невизначеність – 4 методами, що описані у загальних та галузевих міжнародних керівництвах та нормативах. Результати використання комбінації модифікованих карт Шухарта демонструє прийнятність даного підходу для встановлення придатності ретроспективних результатів вимірювання в якості вхідних даних оцінювання невизначеності. Шляхом інтеграції додаткових «меж придатності» до комплекту стандартизованих контрольних меж, даний аналіз набуває можливості одночасного встановлення не лише стабільності, за допомогою інтерпретації геометричних патернів, а й відповідності конкретним критеріям. З використанням даного підходу отримано об'єктивних доказів придатності ретроспективних даних для оцінювання невизначеності вимірювання. Розподіл ретроспективних даних, отриманих в умовах внутрішньо лабораторної відтворюваності, прийнято за нормальне. В результаті оцінювання статистичної контрольованості та відповідності встановленим критеріям було отримано об'єктивні докази придатності даних для оцінки невизначеності. Аналіз інтервалів невизначеності підгруп з обсягом 25 вимірювань, із застосуванням 4 методів оцінювання, продемонстрував значну варіабельність результатів вимірювання біологічних показників безпеки. Інтервал невизначеності міг звужуватися або розширюватися майже на 100% значення у межах п'ятдесяти вимірів. Медіальні значення, отримані із застосуванням різних методів оцінювання невизначеності, становили 2,69%, 22,69%, 39,23 і 43,08%. Дана варіабельність обумовлена обмеженістю охоплення джерел невизначеності, що є притаманним для кожного з проаналізованих методів. Найбільш оптимальним методом показано оцінювання, що базується на рутинному вимірі сертифікованого референсного матеріалу. Ретроспективні результати рутинного вимірювання сертифікованого референсного матеріалу та міжлабораторних порівнянь є ефективним джерелом вхідних даних для оцінювання невизначеності результатів вимірювання показників біологічної безпечності сільськогосподарської сировини. Існує потреба подальшого дослідження для встановлення моделі оцінювання, що надасть можливість врахувати оптимальну кількість впливових джерел невизначеності, які є притаманними для біологічних систем, та не можуть бути одночасно врахованими з використанням існуючих підходів, що пропонуються стандартними методами оцінювання.

Ключові слова: контроль, якість, безпечність, статистика, невизначеність, вимірювання.

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