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ANALYST QUALIFICATION FOR THE ALIQUOT SAMPLING OPERATION UNDER CONDITIONS CLOSE TO ROUTINE ANALYSIS

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Aim. *This study aimed to assess the performance of analysts conducting the standard aliquot sampling operation with a pipette in an inter-laboratory trial under conditions closely resembling routine analytical practices.*
Materials and methods. *Certified 2 mL Mohr pipettes ISO class A; a gravimetric procedure for determining the delivered volume and the corresponding equipment that meets the ISO 4787:2021 requirements; the State Pharmacopoeia of Ukraine statistical approaches.*
Results and discussion. *The testing involved 25 analysts from 6 laboratories performing volume measurements using characterized pipettes in a "blind" experiment (the analyst was unaware of the testing, and the supervisor instructed them to work according to routine procedures). Acceptance criteria were developed based on reliable glassware verification and compliance with the requirements of normal analytical practice (NAP) regarding individual volume deviations from the certified value.*
The average volume deviation did not meet ISO requirements for 40 % of participants (incorrect volumetric glassware verification), individual deviations exceeded NAP requirements for 68 % of participants (incorrect routine analysis performance), and the standard deviation for replicate volume measurements exceeded the requirements for 32 % of participants (unreliable volumetric glassware verification).
Conclusion. *The "blind" testing revealed a significantly higher level of non-compliance compared to traditional proficiency testing (PT). This confirms our assumption that the results of traditional PT reflect the laboratory's best capabilities rather than routine testing practices and cannot be used to assess uncertainty in routine analysis. "Blind" testing provides a more realistic assessment of uncertainty in routine analysis. The high level of non-compliance calls for corrective actions across the entire pharmaceutical sector rather than in individual laboratories*
Keywords: *Mohr's pipette, normal analytical practice, blind testing, inter-laboratory testing, precision, accuracy*

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1. Introduction

The results of any analysis must be of high quality. For quantitative tests, the quality of the results is determined by their uncertainty [1]. In compliance testing (i.e., testing for conformance to specifications [2]), the uncertainty must not exceed a critical value known as the target uncertainty U^{tg}, which is always expressed as an expanded uncertainty, meaning it is an interval, not a standard deviation [3]. Consequently, the reportable result [4] allows for making a reliable decision regarding compliance with specifications [5].

In the pharmaceutical sector, for the uncertainty concept to be effectively applied in compliance testing, the following elements must be implemented [6]:

– a decision rule for determining whether a pharmaceutical product complies with specifications;

– the laboratory must evaluate the actual uncertainty of the analysis results.

The pharmacopeial decision rule, outlined in leading pharmacopoeias [7–9], is based on the following principles:

– analytical variation characteristic of normal (routine) analytical practice (NAP) is taken into account in the specified limits;

– compliance decisions are made solely based on whether the analysis result falls within the specified content limits (CLs). No other tolerances (e.g., those obtained through measurement uncertainty evaluation or setting acceptance and rejection zones) should be applied to the specified CLs.

Despite this, numerous publications exist where authors follow non-pharmacopoeial rules for making decisions on compliance based on different principles for building CLs (e.g. [10]). Such differences introduce unacceptably high risks that decisions regarding compliance with specifications may differ from those made using the pharmacopeial decision rule. Therefore, publications adhering to the pharmacopeial decision rule are crucial for ensuring the consistency and reliability of decisions in the pharmaceutical sector.

Based on the definition of the pharmacopeial decision rule, it can be concluded that a laboratory can correctly perform the analysis if, for all significant factors, the variation does not exceed the value typical for NAP [11]. Therefore, the laboratory should control uncertainty both for the reportable result (combined uncertainty U^{est}) and for its components related to standard analytical operations $\left(U_i^{tg}\right)$. It should be noted that the NAP requirements represent the

"worst case" scenario in which the analysis result still complies with the pharmacopoeial requirements.

Requirements for the maximum permissible uncertainty are established for standard analytical operations $\left(U_i^{tg}\right)$ performed by each laboratory [6]. These include measurement operations and sample preparation operations, such as weighing and dilution [12]. The Technical Guide for the Elaboration of Monographs [13] implicitly provides recommendations for NAP (regarding U_i^{tg}) for dilutions using volumetric flasks and weighing. The State Pharmacopeia of Ukraine (SPhU) explicitly provides recommendations for volumetric flasks, graduated pipettes, and single-mark pipettes (Morh pipettes) [12]. The recommendations of the SPhU are fully incorporated into the WHO guide [6].

Among dilution operations using pharmacopeial volumetric glassware, aliquot sampling with a pipette can be considered the most critical operation [14]. Therefore, information on the actual values of uncertainty for aliquot sampling in the routine analysis is very important as it determines which corrective actions may be necessary. To the best of our knowledge, no articles specifically study the uncertainty associated with aliquot sampling using a pipette.

It is important to note that the values of U_i^{tg} for NAP [15] somewhat exceed the maximum permissible deviations for ISO class A volumetric glassware [16]. This is because, during the verification/calibration of volumetric glassware, multiple volume measurements are conducted, whereas, in routine analysis, the analyst takes an aliquot/dilutes the volume only once. Thus, U_i^{tg} accounts for the typical random variation associated with NAP during a single dilution operation. This increase in uncertainty for standard operations with volumetric flasks is described in [13]. However, this principle is often overlooked in the literature. Based on the NAP recommendations outlined in [13], the SPhU developed the NAP criteria for other types of volumetric glassware [12]. Unfortunately, many authors assess uncertainty in routine analysis by using the maximum permissible deviations for ISO class A volumetric glassware (see, e.g. [17]), which is incorrect. This results in an underestimation of uncertainty for routine analysis and an uncontrolled risk of making incorrect decisions regarding specification compliance. Therefore, publications that apply the NAP approach are greatly needed in the pharmaceutical sector (see, e.g. [18]).

Although the U_i^{tg} requirements (NAP) are somewhat wider than the ISO requirements for class A volumetric glassware, they remain sufficiently strict. If a laboratory does not strive to meet the NAP requirements, there is an unacceptably high risk that the actual uncertainty values for standard analytical operations U_i^{tg} will exceed the NAP recommendations, as has been experimentally confirmed in studies [19, 20].

In an intra-laboratory experiment, a high risk of non-compliance with NAP requirements for aliquot sampling with a pipette was demonstrated [21]. As a result, Proficiency Testing (PT) was organized to monitor the quality of analysts' work with pipettes [14]. It is noteworthy that publications on Proficiency Testing Schemes (PTS) in the pharmaceutical sector are scarce. The few available publications [22] primarily focus on demonstrating that PT was conducted under ISO requirements for PTS [23]. In contrast, as a PTS provider, the Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines uses PT to address metrological issues relevant to the pharmaceutical sector.

In contrast to the previous experiment, which utilized data from the pharmaceutical development stage in an intralaboratory setting, the PT results for aliquoting were highly satisfactory, demonstrating a very low non-compliance rate. This led us to hypothesize that during PT, the analyst may be under pressure and, as a result, act differently than they would in a routine analysis. While the purpose of PTS is to assess the quality of work in routine analysis, it appears that during PT, analysts may perform more diligently, as they would in a responsible analysis. In other words, PT results reflect the best capabilities of the analyst rather than the typical quality of their work in routine analysis.

Therefore, we propose studying an analyst's work quality in an inter-laboratory trial under conditions as close as possible to routine analysis. This approach should help determine whether corrective actions, such as training or seminars, are necessary for individual laboratories only or should be implemented across the entire pharmaceutical sector. It is worth noting that if the quality of an analyst's performance is insufficient, there are unacceptably high risks of non-reproducibility of analytical procedures, which could lead to the release of substandard products onto the market.

To the best of our knowledge, no publications currently address uncertainty in analytical operations or reportable results under conditions that specifically closely resemble routine analysis. Therefore, such studies are of great value to the pharmaceutical sector as they allow for assessing actual values of uncertainty for standard analytical operations $\left(U_i^{est}\right)$, i.e. compliance with the NAP requirements. If U_i^{est} does not exceed the corresponding value of U_i^{tg}, it indicates that the laboratory is capable of providing an acceptable value for U^{est}. This means that the laboratory can make a reliable conclusion regarding compliance with specifications, ensuring that substandard products do not enter the market.

This paper aims to evaluate the performance of an analyst conducting a standard operation of aliquot sampling with a pipette in an inter-laboratory trial under conditions as close as possible to routine analysis.

2. Planning of the research

During the PTS round aimed at assessing the qualification of analysts in aliquot sampling with a pipette, we formulated the key requirements for personnel qualification for routine quality control of pharmaceutical products (key requirements) [14]. This means that the qualification procedure must confirm whether the analyst can reliably perform the following tasks:

1) verify volumetric glassware for compliance with ISO class A requirements;

2) take an aliquot (V_i) with a deviation from the nominal value that does not exceed the requirements of the NAP in routine analysis.

Based on these key requirements, we established criteria for:

1) certification of pipettes used for evaluating analyst qualification [24];

2) assessment of analyst test results.

Since this research also aims to assess analyst qualification against key requirements, it is essential to base this evaluation on well-defined criteria developed for these requirements. However, it is important to consider that different laboratories may perform different numbers of volume measurements depending on their available resources (unlike in PTS, where the number of measurements is standardized for all participants). Therefore, it is advisable to recommend an acceptable number of volume measurements (minimum and maximum) and account for this in the criteria.

To achieve the closest possible approximation to routine analysis practice for analyst test results, an appropriate experimental scheme should be developed.

For comparability with previously obtained results (within the laboratory [21] and in PTS [14]), it is advisable to use the same 2 mL single-mark pipette (Mohr pipette) of ISO class A. According to the results of these studies, it is critical for analyst testing to use certified pipettes with an established certified delivered volume ($\overline{V}_j$, where j is the pipette number) and uncertainty for $\overline{V}_j$ that is acceptable for testing the analyst's compliance with the key analyst qualification requirements [14].

The results should be processed using metrological approaches of the State Pharmacopeia of Ukraine (SPhU) [25, 26].

3. Materials and methods

The testing procedure was developed based on the gravimetric method for determining volume described in ISO-4787:2021 [27]. The developed procedure allows for the determination of the volume delivered by the pipette and for accurate assessment of repeatability and deviation of the volume from the reference value.

Mohr pipettes ISO class A 2 mL, provided by TECHNOSKLO, Czech Republic, and Mohr pipettes ASTM class A 2 mL, provided by KIMBLE DWK LIFE SCIENCE, USA, were used. Other materials included testing liquid, receiver vessel, balances, water thermometer, air thermometer, barometer, and time measurement devices that complied with the requirements of ISO 4787:2021. The SPhU metrological approach was used [25, 26], and the results were analyzed to assess analysts' compliance with the key requirements.

3. 1. Analytical procedure for determining the delivered volume.

The analyst performs aliquoting in the same way as in the routine analysis and weighs an aliquot. All testing conditions are controlled by the supervisor. The provider performs all calculations.

Analysts only perform aliquoting and measure the mass of transferred water:

1. Prior to the test, the pipette, all test equipment, and water shall have stood in the test room for a sufficient time to reach equilibrium with the test room conditions. The room's temperature variation during this time should not exceed 1 °C per hour. The equilibration time is usually about 2 hours, though it can be considerably longer.

Test water should be covered to avoid evaporation cooling.

2. The humidity of the air shall be recorded.

The test room shall have a relative humidity (RH) between 30 % and 80 %.

3. Atmospheric pressure shall be recorded.

4. Air temperature in the room shall be recorded.

The test room shall have a temperature between 17 °C and 25 °C, with a maximum variation of ±1 °C during the test.

5. Water temperature shall be recorded.

The water temperature should be within ±0.5 °C of the air temperature.

6. Transfer a 2 mL aliquot of the testing liquid using a pipette into a pre-weighed receiver (if possible, use the tare function of the balance). To prevent water evaporation, cover the receiver and then weigh it.

7. Proceed as per steps 4–6, repeating the procedures n times ($5 \geq n \geq 15$).

8. Based on the measurement results, calculate the following:

a) delivered volume for each measurement (V_{ij});

b) deviation of the average result of the determined delivered volume $\left(\overline{V_i}\right)$ from the certified value;

c) standard deviation (SD_i) of n repetitive measurements.

The calculated value of the found volume (V_{ij}) is determined under the requirements of ISO 4787:2021.

3. 2. Statistical data processing.

The results should be processed according to the SPhU approaches [12, 25, 26].

4. Results

Further in the text, we refer to uncertainty as the expanded uncertainty for a 95 % confidence level when calculated from experimental data. This expanded uncertainty corresponds to a one-sided confidence interval of 95 %.

4. 1. Characterization of pipettes

Pipettes used for laboratories in Ukraine were the same as for PTS [14]. For the U.S. laboratory, the pipette was calibrated by an analyst qualified by the PTS provider to calibrate pipettes designated as test items (TI) for testing the analyst's compliance with the key requirements [14]. For all pipettes, the certified value (V_{Cert}) was insignificantly different from the nominal value at a 95 % confidence level [6, 25] under ISO requirements for class A glassware [16]:

$$\left|2 - V_{Cert}\right| \leq 0.01 \text{ mL} \times 0.32 = \\ = 0.0032 \text{ mL or } 0.16\ \%. \quad (1)$$

Therefore, the nominal value was accepted as the certified value.

The uncertainty for V_{Cert} for the pipette (as a precision component, Δ_v), certified for the U.S. laboratory, was:

$$\Delta_V = RSD_{Cert} \times \times \frac{t\left(n-1=14;\ 95\ \%;\ \text{one-sided}\right)}{\sqrt{15}} = 0.028\ \%, \qquad (2)$$

where RSD_{Cert} represents the relative standard deviation for the delivered volume obtained during the certification of the pipette;

15 is the number of measurements performed during the pipette certification process.

Δ_v is insignificant compared to the ISO requirements according to equation (1).

Thus, all pipettes were suitable for testing analysts' compliance with the key requirements following the methodology we formulated [14, 24].

4. 2. Development of the experiment design

To bring the testing as close as possible to the normal laboratory practice, a "blind" experiment format was proposed, i.e. the supervisor involved the analysts engaged in routine analyses, instructing them to take aliquots in exactly the same way as in routine analyses, ensuring that they were unaware of the purpose of the task, i.e. performing qualification, which eliminated the increased pressure on analysts that may arise in PTS due to the heightened responsibility. Based on the study objectives, no prior specific training/testing of the analysts in the laboratory was carried out.

The delivered volume was determined using the gravimetric method in accordance with ISO 4787 [27], as also applied in PTS [14]. This method requires monitoring parameters that are not typically controlled in routine analysis, including air humidity and atmospheric pressure (recorded at the beginning of the experiment) and temperature variation of air and water (monitored during testing). To closely simulate routine analysis conditions, all additional controls were performed by the supervisor. The analyst only measured the aliquot mass following the analytical procedure. Unlike in PTS, where participants calculate results as part of their responsibilities per the key requirements, all subsequent calculations in this study were carried out by the testing provider.

4. 3. Establishing evaluation criteria for analyst performance

It was proposed that the same criteria be used for evaluating results during PTS, as these criteria are based on the key requirements [14]. However, consideration was given to the possibility of using a different number of repetitive volume measurements in different laboratories.

The recommended number of volume measurements (n) for "blind" testing ranges from 5 to 15. An n value of 5 is recommended as the minimum number of measurements for verifying Mohr pipettes, which reliably ensures the required uncertainty for Mohr pipette verification purposes [15]. An n value greater than 15 is less practical as, based on our experience, the analyst begins to fatigue, creating a high risk of deviation from repeatability conditions. EURACHEM also does not recommend using more than 15 measurements, as it does not lead to a significant reduction in uncertainty for the reportable result [1].

The following parameters were evaluated for volume measurement results.

Parameter A: Bias. Deviation of the average result of the determined delivered volume $\left(\overline{V}_i\right)$ from the nominal value of 2 mL (certified value):

$$Criterion\ a: \overline{d}_i = \left|\overline{V}_i - V_{Cert}\right| \le U^{tg}_{ISO};$$

$$\overline{d}_i \le 0.010\ \text{mL or}\ 0.50\ \%. \qquad (3)$$

This parameter is used because the analyst performing routine analyses should be able to verify the volumetric glassware correctly.

Parameter *a* is used equally for $5 \le n \le 15$.

Parameter B: *NAP compliance for individual deviations*. Individual deviations of the delivered volume (V_{ij}) from a nominal value of 2 mL:

$$\text{Criterion } b\text{: for any } V_{ij}: d_{ij} = \left|V_{ij} - V_{Cert}\right| \le U^{tg}_{NAP},$$

$$d_{ij} \le 0.012\ \text{mL or}\ 0.61\ \%, \qquad (4)$$

where U^{tg}_{NAP} is the maximum permissible uncertainty for working with a 2 mL ISO class A pipette, as per the SPhU [12] and WHO [6] recommendations for NAP requirements.

Parameter *b* also applies equally for $5 \le n \le 15$.

Parameter C: Precision. Standard deviation (*SD*) of 5–15 repetitive determinations of delivered volume.

The obtained *SD* must provide a confidence interval that it is insignificant compared to the ISO requirements for the verification of 2 mL pipette class A [15]. Therefore, the requirements for *SD* (SD_{crit}) depend on the number of measurements.

The requirements were calculated according to the formula:

$$SD_{crit} \times \frac{t\left(\text{one sided, } 95\ \%,\ f = n-1\right)}{\sqrt{n}} = = 0.010\ \text{mL} \times 0.32 = 0.0032\ \text{mL}, \qquad (5)$$

or 0.16 %;

$$SD_{crit} = \frac{0.0032\ \text{mL} \times \sqrt{n}}{t\left(\text{one sided, } 95\ \%,\ f = n-1\right)}.$$

For n=5:

$$SD_{crit} = U^{tg}_{ISO} \times 0.32 \times \frac{\sqrt{5}}{t\left(\text{one sided, } 95\ \%,\ f = 4\right)} = = 0.010\ \text{mL} \times 0.32 \times \frac{\sqrt{5}}{2.1318} = 0.0034\ \text{mL}.$$

For n=10:

$$SD_{crit} = U_{ISO}^{tg} \times 0.32 \times \frac{\sqrt{10}}{t(\text{one sided, 95 \%, } f = 9)} =$$

$$= 0.010 \text{ mL} \times 0.32 \times \frac{\sqrt{10}}{1.833} = 0.0055 \text{ mL}.$$

4. 4. Organization of the experiment

The provider supplied one TI to the supervisors of each Ukrainian participant laboratory, whereas the pipette calibration for the U.S. laboratory was performed independently. Each analyst from a participating laboratory was required to perform n measurements, depending on the laboratory's resources. The results were then reported to the provider, who carried out all calculations as specified in Section 3. 1.

4. 5. Evaluation of "blind" testing result

"Blind" testing was organized for quality control laboratories of manufacturers of pharmaceutical dosage forms. The study included 5 laboratories from Ukraine and 1 laboratory from the USA, with a total of 25 participating analysts. The Ukrainian laboratories performed 10 measurements of the delivered volume, while the U.S. laboratory conducted 5 measurements.

The results were classified as follows (as shown in Tables 1–3):

– compliance: results meeting the criterion (transparent background);

– noncompliance: results exceeding the criterion by no more than 32 % (light grey background);

– critical noncompliance: results exceeding the criterion by more than 32 % (dark grey background).

This classification of uncertainty assessment is due to the fact that for a 95 % confidence level, a deviation of 32 % is considered insignificant [12]. Therefore, such a deviation can be explained by random factors. A deviation of more than 32 % indicates that the analyst has a problem with the operation of taking an aliquot, which cannot be explained by random factors.

Table 1 presents the combined results for laboratories according to criterion *a* (*bias* for $\overline{V}_i$), that is, the analyst's ability to correctly verify pipettes for compliance with ISO class A. Table 2 shows the results of the maximum deviations of individual values of the delivered volume according to criterion *b* (*bias* for V_{ij}), i. e. the analyst's ability to correctly perform routine analysis following NAP requirements. Table 3 provides the *SD* precision results for the delivered volume. This criterion ensures the high reliability and correctness of the verification of pipettes (insignificance of the precision component for the uncertainty of the verification).

The results shown in Table 1 indicate noncompliance in 40 % of cases and critical noncompliance in 28 % of cases. The maximum deviation exceeds the ISO requirement by 192 %.

Table 1

Deviation of the average delivered volume from the certified value for the pipette.
Criterion *a* (*bias*) : $\overline{d}_i = \left|\overline{V}_i - V_{Cert}\right| \le 0.01 \text{ mL } (0.5 \%)$

Laboratory	Analyst	Compliance	Bias, % from U_{ISO}^{tg} *
1	1	+	84
	2	–	164
	3	+	33
	4	–	141
2	5	–	173
	6	–	154
	7	–	132
3	8	+	5
4	9	+	34
	10	+	50
	11	+	68
	12	+	39
	13	+	15
	14	+	6
5	15	–	134
	16	+	34
	17	+	9
	18	+	10
	19	+	51
	20	+	17
6	21	–	192
	22	–	110
	23	+	9
	24	–	101
	25	–	140

Note: * – $Bias, \% = \overline{d}_i / U_{ISO}^{tg} \times 100$.

Table 2

Deviation of individual values of the delivered volume from the certified pipette volume.
Criterion *b* (NAP compliance for individual deviations): $d_{ij} = \left|V_{ij} - V_{Cert}\right| \le 0.012$ mL (0.61 %)

Laboratory	Analyst	Rate of non-compliance	%Max deviation from NAP*
1	1	2	117
	2	9	157
	3	0	86
	4	9	134
2	5	9	162
	6	8	155
	7	7	162
3	8	0	45
4	9	0	90
	10	0	85
	11	1	113
	12	1	118
	13	2	112
	14	0	64
5	15	7	139
	16	1	172
	17	0	65
	18	0	39
	19	3	247
	20	1	144
6	21	4	240
	22	2	142
	23	0	31
	24	1	102
	25	4	160

*Note: * – %Max deviation from NA=d_{ij}/0.012* mL×100 %.

Table 3

Precision for delivered volume. Criterion *c* (precision) $SD \le SD_{crit}$

Laboratory	Analyst	Compliance	% from SD_{crit}*
1	1	+	78
	2	+	42
	3	+	80
	4	+	53
2	5	+	45
	6	+	52
	7	+	63
3	8	+	47
4	9	+	92
	10	+	59
	11	+	66
	12	–	128
	13	–	153
	14	+	82
5	15	+	41
	16	–	133
	17	+	72
	18	+	42
	19	–	247
	20	–	124
6	21	–	231
	22	–	172
	23	+	74
	24	+	73
	25	–	109

*Note: * – % from SD_{crit}=SD_i/SD_{crit}×100 %.*

The results shown in Table 2 indicate noncompliance in 68 % of cases, and critical noncompliance in 48 %. The maximum deviation exceeds the NAP requirement by 247 %.

The results presented in Table 3 show noncompliance in 32 % of cases and critical noncompliance in 20 % of cases.

5. Discussion

The variation associated with aliquoting can significantly impact the quality of reportable results. However, previous studies have not investigated the actual uncertainty associated with this standard operation when performed under routine analysis conditions in pharmaceutical quality control laboratories. Similarly, the compliance of aliquoting practices with NAP requirements under routine analysis conditions remains unexplored despite its critical role in ensuring the reproducibility and reliability of analytical procedures between laboratories [6]. Consequently, the findings of this study are of considerable practical importance, particularly for the pharmaceutical sector.

The following results were reported in PTS [14]: among 64 analysts, noncompliance with criterion (*a*) was observed in 2 analysts (3 %), criterion (*b*) in 3 analysts (5 %), and criterion (*c*) in 1 analyst (1.5 %). In contrast, a prior intra-laboratory experiment [21] involving routine analyses with aliquot weighing revealed a significantly higher rate of noncompliance with NAP requirements, observed at 21 %.

It is evident that the results of "blind" testing differ significantly from those of the PTS, revealing a notably high rate of noncompliance across all criteria compared to the exceptionally low rates observed in the PTS. Moreover, the "blind" testing results are consistent with the previous studies conducted under routine analysis conditions [21]. This confirms our hypothesis that, during PTS, analysts experience pressure and perform measurements as a responsible analysis rather than as routine work. Consequently, PT results are not always suitable for assessing uncertainty in routine analysis, which contrasts with the recommendations of the OMCL [28].

Some official documents (e.g., [29]) recommend conducting internal PTS. However, if internal PTS is organized similarly to external PTS, the results can reflect the analyst's best capabilities rather than the quality of their routine work. In contrast, "blind" testing offers significant advantages by yielding results that accurately reflect the quality of routine analyses, making it a reliable method for assessing the actual uncertainty of the reportable result.

It should be noted that the United States Pharmacopeia (USP) relies on a different standard for volumetric glassware, specifically the ASTM standard [30]. In this standard, the requirements for Mohr pipettes (referred to as Transfer Pipets in the USP) are somewhat stricter than those of ISO class A. For example, the maximum permissible deviation from the nominal volume for a 2 mL pipette is 0.006 mL (0.30 %) under the ASTM standard, compared to 0.01 mL (0.50 %) under ISO. However, we believe that NAP requirements should remain consistent regardless of the volumetric glassware standard used by the analyst. The NAP requirements for Mohr pipettes [6, 12] are derived from the ISO's standard's maximum permissible deviation from the nominal volume. However, these requirements serve as limit values, making adopting stricter standards practically unattainable. Since NAP requirements must be adhered to for any aliquoting operation in any laboratory, they become more stringent in practice. Therefore, it is rational to apply the proposed NAP requirements irrespective of the volumetric glassware standard employed.

The results of "blind" testing revealed that their quality is strongly influenced by the laboratory, as each laboratory has a distinct level of knowledge and practical skills that are generally consistent among its analysts.

Corrective actions. According to the SPhU approach [26], at a 95 % confidence level, no more than 5 out of 25 analysts and 3 out of 10 aliquot outliers may fail to meet the requirements due to random variation in results (binomial distribution). Unfortunately, this approach is not suitable for assessing only 5 measurements, as the uncertainty of such an estimate becomes unacceptably large. If these requirements are met, corrective actions are only necessary for laboratories where noncompliance was identified. Conversely, if the requirements are not met, corrective actions are required for the pharmaceutical sector as a whole. Therefore, "blind" testing indicates the need for further training and testing across the pharmaceutical sector to ensure the standard operation of aliquoting is of adequate quality.

Practical relevance. The results obtained clearly highlight the necessity for corrective actions across the entire pharmaceutical sector, including the organization of specialized workshops, as well as analyst training and testing.

Furthermore, when PTS results are used to estimate the uncertainty of a reportable result for routine analysis, it is essential to consider the risk that these results may significantly underestimate the actual uncertainty.

Research limitations. The proximity of measurement conditions in a "blind" experiment to those of routine analyses is subjective and largely determined by the supervisor's ability to ensure that the analyst performs as they would in routine scenarios. However, we are unaware of alternative approaches that can effectively simulate routine analysis conditions during analyst testing. In our experiment, "blind" testing yielded very promising results.

Prospects for further research. The proposed "blind" testing approach can be applied to any PTS tests. The comparison of PTS results with "blind" testing ones provides valuable insights into the relationship between the quality of work in responsible and routine analyses. Thus, conducting "blind" testing for other individual standard analytical operations, as well as for

integral tests that assess uncertainty for the entire analytical system (see, e.g., [26], Section 7. 6. 3. Integral Test for Verification of the Analytical System and Personnel Qualification for Spectrophotometry Method), holds significant potential for improving the overall quality of analytical practices.

Another consideration is ensuring compliance with NAP requirements when performing a large number of repetitive measurements. If the population of measurement results follows a Gaussian distribution (as expected), an increase in the number of measurements also increases the probability of obtaining results that significantly deviate from the true value (μ), even though the overall mean value remains the closest estimate of μ [26]. Therefore, we believe that for a large number of measurements (more than 15), the NAP criteria should be modified to allow for wider deviations, similar to the approach used for the Uniformity of Dosage Units test when dealing with a large number of measurements [31].

6. Conclusion

The "blind" testing in the inter-laboratory experiment confirmed that PTS results should be regarded as reflecting the laboratory's best capability rather than routine testing practice. "Blind" testing provides a more accurate assessment of an analyst's performance under conditions that closely resemble routine analysis.

The results suggest that corrective actions are needed for the pharmaceutical sector as a whole rather than for individual laboratories.

The real situation can be assessed, for example, when an analyst's qualification is evaluated in an intra-laboratory PTS. Such a PTS should have a special format to bring the qualification conditions as close as possible to routine analysis conditions. Such a format can be a "blind" test for the analyst.

A special format is needed to evaluate the real situation, such as assessing an analyst's qualifications in an intra-laboratory PTS. This format should aim to replicate routine analysis conditions as closely as possible. "Blind" testing of the analyst could be an effective approach to achieve this.

Conflict of interests

The authors declare that they have no conflict of interests in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available at a reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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