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Professional paper

Use of measurement uncertainty for determination of calibration frequency of the volumetric equipment

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Abstract

Analytical laboratories should confirm the traceability of volumetric measurements used in support of testing and calibration activities, by establishing procedures for calibration and check of the laboratory glassware used in volumetric measurements. Different national accreditation bodies establish different minimum requirements for the volumetric glassware used in a laboratory, regarding the validity of the calibration and the frequency for the verification of the suitability of the volumetric glassware. The intervals, methods, and specification criteria for qualification of volumetric glassware usually are established by each laboratory, taking into account the type of equipment following the relevant standard requirements.

We have estimated the measurement uncertainty of the volumetric equipment that is mostly used in the laboratory for quality control of medicines (volumetric flasks, single-volume pipettes, and burettes), and identified that the temperature effect and meniscus reading (precision and accuracy) are the most contributing sources in the uncertainty. Additionally, we have performed statistical evaluation by comparing the obtained results with the calibration certificate values and with the experimental results from qualification in previous years. Based on the obtained results it was concluded that the defined frequency of calibration once a year can be extended, as long as the measurements are performed according to the standard operating procedures and in strictly controlled environmental conditions.

Key words: volumetric equipment, verification, measurement uncertainty, calibration frequency.

Introduction

Volumetric glassware comprises a group of measuring equipment used for direct measurement and has a significant influence on the validity of the testing result. Additionally, the volumetric accuracy is critical for the performance of the method. Volumetric glassware used in the analytical laboratory may either deliver volume (burettes and bulb burettes, single volume pipettes, one-mark pipettes, bulb burettes, graduated pipettes, including blow-out pipettes) or contain a stated volume (one-mark volumetric flasks, graduated measuring cylinders). The class A and AS volumetric glassware, used in the laboratory is supplied with a batch calibration certificate, where the tolerance and the error of the material are traceable to an international standard (UKAS, 2009).

The laboratory performing analytical testing must have a procedure for management (handling, storage, use, and maintenance) and establish a calibration program for the measuring equipment (ISO/IEC 17025:2017). Before use, measuring equipment must be calibrated and verified that conforms to the laboratory's requirements for its measurement accuracy and measurement uncertainty. To keep the confidence in the status of calibration and performance of the equipment a calibration program and a

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plan for intermediate checks must be established (ISO/IEC 17025:2017).

Qualification of equipment refers to actions of proving and documenting that any analytical equipment complies with the required specifications and performs suitably for its intended purpose. The performance of volumetric equipment should be verified at appropriate intervals according to a plan and procedures established by the laboratory, taking into account the type of equipment, the extent of use and supplier's recommendations (WHO, 2010).

The initial level of the qualification of the volumetric glassware should include a selection of the volumetric glassware that is manufactured according to the requirements of the corresponding ISO standards (ISO 385, ISO 648, ISO 1042, and ISO 4787).

The second level of the qualification of the volumetric glassware should include the confirmation of compliance with the laboratory's requirements and with the relevant standard specification. This is done by performing a visual inspection of the glassware to check for scratches, blurred, broken or chipped glass, and calibration marks (verify the compliance with marking requirements), as well as checking the batch calibration certificate of the supplied equipment. In the cases where the certificate is not available, calibration of the volumetric glassware should be performed before use.

The need for the calibration of the laboratory glassware before first use depends on the volumetric accuracy that is critical for the performance of the method, for which the glassware is intended to be used. The criteria for performing the calibration of the volumetric glassware, regarding the maximum permissible overall error in the testing method and maximum specified tolerance for the volumetric glassware should be established by each laboratory following the requirements of the corresponding ISO standards (ISO 385, ISO 648, ISO 1042 and ISO 4787).

Calibration of the laboratory glassware may be performed internally by the authorized (experienced and trained) personnel, according to the suitably defined procedure, developed by the laboratory, or externally by the accredited institution.

According to ISO 4787, the general procedure for calibration of laboratory glassware is based on a gravimetric method, that includes the determination of the volume of water, either contained in or delivered by the volumetric equipment.

The third level of the qualification of the volumetric glassware should include verification of the calibration status, by performing calibration on the glassware. The calibration intervals and specification criteria should be based on their type and characteristics as well as the frequency of their use, following the relevant standard requirements. It is recommended to define the calibration frequency for at least once a year or to extend these intervals if suitably justified (OMCL, 2022).

The fourth level of the qualification of the volumetric glassware should include visual inspection of the glassware before each use and the deteriorated material must be removed if it would compromise the safety of personnel or the purposes for which it is intended. In the case of aggressive use of volumetric equipment, such as the use of hydrofluoric acid or other corrosive substances, thermal or mechanical shock, visual inspection should be performed after cleaning and before storing the glassware.

The measurement uncertainty of the volumetric equipment and confidence in the calibration status are directly connected with the performance of the equipment (Lorefice, 2009). Therefore, the estimation of the measurement uncertainty is a necessary step of the verification procedure.

The aim of this study was to estimate the measurement uncertainty of the volumetric glassware that is mostly used in the laboratory for quality control of medicines (volumetric flasks, single-volume pipettes, and burettes) and to identify the most contributing source of the uncertainty. Additionally, we have performed the statistical evaluation by comparing the obtained results with the calibration certificate values and with the experimental results from the qualification done in the previous years, to define the reduced frequency of calibration.

Material and methods

Materials

Water deionized (TKA-LAB Reinst-wassersystem), with high purity and free from dissolved gases, with the specific conductivity of 1×10^{-4} S × m^{-1} at 20 °C was used for the calibration of the volumetric glassware. Temperature of the water was controlled using a calibrated thermometer with error not exceeding ± 0.1 °C. Measurements were performed on the Sartorius MSE125P-1CE-DU balance (d = 0,00001 g/0,0001g (0,01 mg/0,1 mg)), e = 0,001g (1 mg) supplied by Sartorius AG GOTTINGEN Germany.

Volumetric equipment (volumetric flasks, single-volume pipettes, and burettes) was supplied from ISOLAB, Bulb. The values for the tolerance limits, average value and standard tolerance obtained from the calibration certificates are given in Table 1.

Qualification procedure

Qualification of the selected volumetric glassware (Table 1) was performed according to the internal procedure of the Center for Drug Quality Control, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje. The ambient temperature was controlled using calibrated temperature loggers and the volumetric glassware subject of calibration was cleaned, dried, and inspected visually before use. The temperature of the purified water used for volume measurements was accurately measured and recorded. After weighing, the empty volumetric glassware

Table 1. Tolerance limits and calibration results for the volumetric equipment

	Tolerance limits	Average value	Standard Tolerance
One-mark pipettes			
1 mL	1.000 mL+/-0.008 mL	1.003 mL	0.0015 mL
2 mL	2.000 mL+/-0.010 mL	2.003 mL	0.0036 mL
5 mL	5.000 mL+/-0.030 mL	5.004 mL	0.0037 mL
10 mL	10.000mL+/-0.020 mL	10.005 mL	0.0025 mL
Volumetric flasks			
5 mL	5.000 mL+/-0.025 mL	5.002 mL	0.0044 mL
10 mL	10.000 mL +/-0.025 mL	10.006 mL	0.0062 mL
20 mL	20.000 mL +/-0.040mL	20.004 mL	0.0090 mL
25 mL	25.000 mL +/-0.040mL	25.003 mL	0.0079 mL
50 mL	50.000 mL +/ 0.060mL	49.986 mL	0.0113 mL
100 mL	100.000 mL +/-0.100mL	99.985 mL	0.0099 mL
Burettes			
25 mL	25.000 mL+/-0.030 mL	25.03 mL	0.011 mL
50 mL	50.000 mL+/-0.050 mL	50.004 mL	0.0045 mL

was placed on a flat horizontal surface, filled with water up to the mark, and visually inspected (the outer and inner surfaces above the water level for dryness and for the presence of bubbles). The mass of the water contained in the flasks/delivered by the pipettes and burettes was measured. The procedure was repeated 5 times for the volumetric flasks, and 10 times for burettes and the pipettes. Calculations and statistical evaluation were performed using Microsoft Excel 2013.

Results and discussion

The volume of the water contained in or delivered from the volumetric equipment in mL, was calculated from the weighed mass m (g), multiplied by the conversion factor Z, which values (mL/g) as a function of temperature and pressure were obtained from the existing literature (Win, 2021). Standard deviation (S) and relative standard deviation (RSD) were calculated from the results for each series of measurements.

$$V = m * Z$$

The relevant sources of uncertainty were identified and are presented in a cause-and-effect diagram, shown in Fig. 1.

Considering that the instruments used for the temperature and barometric pressure reading are calibrated and used according to the standard operating procedures, the contribution of the uncertainty of the factor Z compared to the other components is extremely small and can be considered negligible (Win, 2021).

To identify the major source of the uncertainty of the volume, the standard uncertainty of each source was estimated and taken into account for the calculation of the relative combined standard uncertainty, $\frac{u(V)}{V}$, using the following formula:

$$\frac{u(V)}{V} = \sqrt{(\frac{u(V_m)}{m})^2 + (\frac{u(V_T)}{V})^2 + (\frac{u(V_{cal})}{V})^2 + (\frac{u(V_{rep})}{V})^2 + (\frac{u(V_{acc})}{V})^2}$$

where: $\frac{u(Vm)}{m}$ is relative uncertainty of the weighed

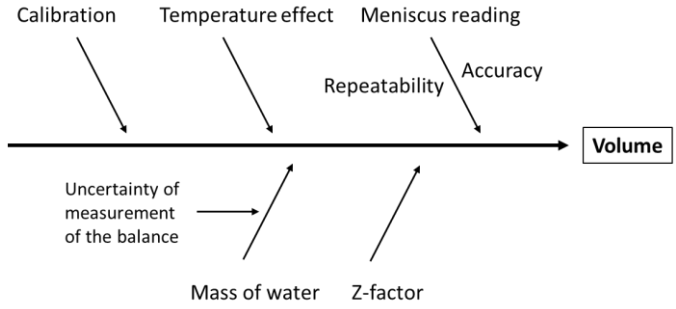

Fig. 1. Cause-effect diagram of the uncertainty sources of the measured volume.

mass, $\frac{u(V_T)}{V}$ is the relative uncertainty of the temperature effect, $(\frac{u(V_{cal})}{V})^2$ is the relative uncertainty of the calibration stated on the manufacturer's calibration certificates, $(\frac{u(V_{rep})}{V})^2$ and $(\frac{u(V_{acc})}{V})^2$ are the contributors to the uncertainty of the meniscus reading (repeatability and accuracy) (Kadis, R., 2004).

The standard uncertainty of measurement of the weighed mass $u(V_m)$ was calculated from the uncertainty of measurement of the weighing result stated on the balance calibration certificate, as the uncertainty of balance use, with coverage facor, k = 2, for approximately 95 % level of confidence (OMCL Guideline, 2020).

The uncertainty of measurement of the volumetric glassware, at a temperature of 20ºC, was stated as standard tolerance, in the calibration certificate of the corresponding batch of volumetric flasks, single-volume pipettes and burettes used, without any information about the level of confidence and distribution. Calculations were performed assuming triangular distribution considering that the actual volume is more likely to be at the center than at the extremes of the range (Elison and Williams, 2012).

$$u(V_{cal}) = \frac{u(V_{calibration\ certificate})}{\sqrt{6}}$$

Taking into account that the volumetric flask has been calibrated at 20 ºC, the uncertainty from the temperature effect was calculated using the temperature difference (T°C = recorded temperature for each measurement – 20 ºC) and the coefficient of volume expansion, i.e., 0.00021 per 1 ºC, assuming rectangular distribution (Elison and Williams, 2012).

$$u(V_T) = \frac{\mathrm{V(mL)} \cdot \mathrm{T^oC} \cdot \frac{0.00021}{1^oC}}{\sqrt{3}}$$

Expanded uncertainty was calculated by multiplying the combined standard uncertainty with the coverage factor, corresponding to the number of conducted single measurements, for approximately 95 % level of confidence. The results are summarized in Table 2.

The estimates for standard uncertainty of each individual source were used for evaluation of the contributions of each uncertainty source to the combined standard uncertainty (Fig. 2).

From the Fig. 2 it is evident that the major contributor to the combined standard uncertainty is the uncertainty arising from the temperature effect (64.17%), followed by the contribution of the meniscus reading (precision 14.05% and accuracy 12.30%), and the uncertainty of the calibration (9.41%). The uncertainty contributions of the weighed mass is less than one third of the major contributor, and can be disregarded (Elison and Williams, 2012).

The experimental results were statistically evaluated by comparing the obtained values to the values from the manufacturers calibration certificate using student t - test and F-test for variances (Table 3). There was no statistically significant difference of the experimental values, compared to the values stated in the manufacturers certificate of quality for the volumetric glassware for a confidence level of 95% ($p = 0.05$). The calculated T and F-values were below the critical values; T = 2.26 (Two - tail, $p = 0.05$, 9 degrees of freedom), T = 2.78 (Two - tail, p=0.05, 4 degrees of freedom), F=3.18 (confidence level 95%, 9 degrees of freedom and F=6.59, confidence level 95%, 4 degrees of freedom), except for the T values for the one-mark bulb pipettes of 1 mL and 5 mL, where the T values are higher than the T-critical (Table 3). Taking into account that the experimentally obtained average values, together with the estimated measurement uncertainty are within the tolerance limits (Table 2), the qualification results are considered acceptable and the volumetric flask are suitable for the laboratory use.

Additionally, the experimental results were statistically evaluated using statistical F and T tests, with the results from the conducted measurements during periodical annual qualification in the previous years (2019, 2020, 2021 and 2022). Summary of the results from statistical evaluation of the experimental values versus previous experimental values is given in Table 4. The results from the qualification of the one - mark bulb pipettes for the year 2019 were not available.

Table 2. Summary of the experimental results with estimated measurement uncertainty

Nominal volume	Average volume (mL)	U (mL)
One-mark pipettes		
1 mL	1.004	0.003
2 mL	2.004	0.006
5 mL	5.006	0.006
10 mL	10.005	0.011
Volumetric flasks		
5 mL	5.005	0.013
10 mL	10.002	0.024
20 mL	20.004	0.034
25 mL	25.006	0.044
50 mL	49.986	0.070
100 mL	99.980	0.170
Burettes		
25 mL	25.002	0.034
50 mL	50.008	0.070

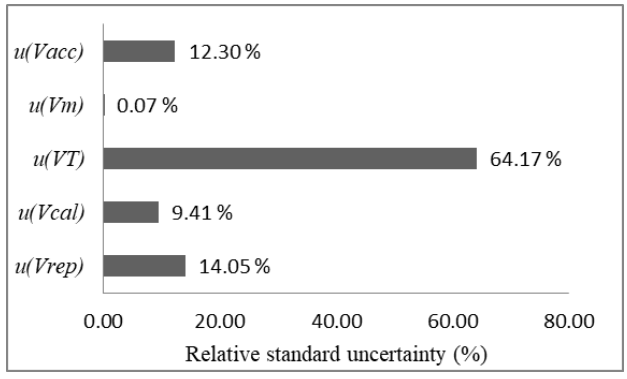

Fig. 2. Contribution of each uncertainty source to combined standard uncertainty.

Table 3. Summary of the results from statistical evaluation of the experimental values versus calibration certificate values

One-mark bulb pipettes	1 mL	2 mL	5 mL	10 mL		
T Value	4.40	1.80	2.50	1.05		
F Value	1.16	1.95	0.67	0.86		
Volumetric flasks	5 mL	10 mL	20 mL	25 mL	50 mL	100 mL
T Value	2.46	1.68	0.14	1.62	0.02	1.89
F Value	1.02	2.08	2.63	0.82	0.63	0.76
Burettes	25 mL	50 mL				
T Value	0.96	1.45				
F Value	2.26	2.67				

Table 4. Summary of the results from statistical evaluation of the experimental values versus previous experimental values

One-mark bulb pipettes	1 mL	2 mL	5 mL	10 mL		
2023/2022						
T Value	1.33	4.28	5.83	2.33		
F Value	0.60	0.44	5.66	0.03		
2023/2021						
T Value	0.31	0.97	0.94	0.68		
F Value	0.02	0.14	1.73	0.02		
2023/2020						
T Value	1.66	1.82	4.16	0.68		
F Value	0.11	0.37	4.78	0.02		
Volumetric flasks	5mL	10mL	20mL	25mL	50mL	100mL
2023/2022						
T Value	2.34	0.01	1.56	1.08	1.11	3.22
F Value	0.01	0.00	0.04	0.00	0.01	0.00
2023/2021						
T Value	1.28	0.50	0.04	0.30	0.45	0.99
F Value	0.03	0.06	0.07	0.02	0.01	0.00
2023/2020						
T Value	4.82	0.03	1.97	-1.08	0.30	2.13
F Value	0.05	0.03	0.06	0.00	0.00	0.00
2023/2019						
T Value	1.85	1.08	3.73	1.08	1.86	2.39
F Value	0.01	0.90	0.80	0.32	0.03	0.00
Burettes	25 mL	50 mL				
2023/2022						
T Value	4.05	5.03				
F Value	0.35	2.27				
2023/2021						
T Value	4.76	5.18				
F Value	0.02	0.09				
2023/2020						
T Value	3.28	0.11				
F Value	0.02	0.02				
2023/2019						
T Value	7.45	3.79				
F Value	0.02	0.29				

All the obtained F values were below the F critical value, indicating that the measurements are performed with a good precision, and that the measurement uncertainty is constant and well controlled. The statistically significant difference found in some of the results may be due to the difference in the ambient conditions for performing the qualification and different batches of the volumetric equipment used in different years.

Based on the obtained results, it can be recommended to extend the time interval for the verification of the calibration status of the volumetric instrument for a period greater than one year.

Conclusion

The laboratories performing testing activities must verify that the volumetric equipment used meet the requirements for its measurement accuracy and measurement uncertainty by using suitably calibrated equipment and planning and performing periodical qualification to keep the confidence in its performance. Based on the obtained results it was concluded that the defined frequency of calibration once a year can be extended, as long as the measurements are performed according to the standard operating procedures and in strictly controlled environmental conditions.

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Резиме

Примена на мерната неодреденост за определување на фреквенцијата на калибрација на волуметриската опрема

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Клучни зборови: волуметриска опрема, верификација, мерна неодреденост, фреквенција на калибрација.

Аналитичките лаборатории треба да ја потврдат следливоста на волуметриските мерења што се користат при спроведување на активностите за тестирање и калибрација, преку воспоставување процедури за калибрација и проверка на лабораториската опрема што се користи при волуметриските мерења. Различни национални тела за акредитација воспоставуваат различни минимални барања за волуметриската опрема, што се користи во лабораторија, во однос на валидноста на калибрацијата и фреквенцијата на верификацијата на соодветноста на волуметриските стаклени садови. Интервалите, методите и критериумите за за квалификација на волуметриските стаклени садови обично ги утврдува секоја лабораторија, земајќи го предвид типот на опремата, во согласност со барањата на релевантните стандарди.

Во овој труд, утврдена е мерната неодреденост на волуметриската опрема, што најчесто се користи во лабораторијата за контрола на квалитетот на лековите (волуметриски колби, пипети со еден волумен и бирети) и како најзначајни извори на мерна неодреденост се идентификувани температурниот ефект и отчитувањето на менискусот (прецизноста и точноста). Дополнително, извршена е статистичката евалуација на резултатите преку споредба на добиените резултати со вредностите на сертификатот за калибрација и со експерименталните резултати од квалификацијата, спроведена во претходните години. Врз основа на добиените резултати беше заклучено дека дефинираната фреквенција на калибрација (еднаш годишно), може да се продолжи, доколку мерењата се вршат според стандардните работни процедури и во строго контролирани услови на животната средина.