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Determination of uncertainty for volume measurements of a piston-operated volumetric apparatus using a photometric method

Détermination de l'incertitude de mesure pour les mesurages volumétriques des appareils volumétriques à piston au moyen de la méthode photométrique

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 48, *Laboratory equipment*.

This second edition cancels and replaces the first edition (ISO/TR 16153:2004), which has been technically revised.

The main changes are as follows:

— the term “standard deviation of the mean delivered volume” has been replaced in this document by “repeatability” according to ISO/IEC Guide 99 (VIM);

— a new uncertainty calculation example has been supplied;

— new uncertainty components have been added, namely, reproducibility, air cushion and resolution;

— new Annex A concerning the uncertainty in use of a single delivered volume has been added;

— new Annex B concerning volume correction due to pressure changes has been added.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The example given in this document is informative and supports the requirements found in ISO 8655-8:2022, 9.4 and ISO 8655-7:2022, 4.2, to perform an estimation of measurement uncertainty when calibrating POVA according to the measurement procedures described in these documents and the principles of ISO/IEC Guide 98-3.

The revision of this document coincides with a major revision of the ISO 8655 series in 2022, reflecting the state-of-the-art measurement procedures and approaches for the estimation of measurement uncertainty.

Determination of uncertainty for volume measurements of a piston-operated volumetric apparatus using a photometric method

1 Scope

This document gives detailed information regarding the evaluation of uncertainty for the photometric reference measurement procedure specified in ISO 8655-8 and the photometric procedure specified in ISO 8655-7:2022, Annex B according to ISO/IEC Guide 98-3.

This document also describes the determination of other uncertainty components related to the liquid delivery process of a piston-operated volumetric apparatus (POVA), e.g. repeatability and handling. Furthermore, it provides examples for the calculation and application of the uncertainty of the mean delivered volume and the uncertainty in use of a single delivered volume.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8655-1, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8655-1 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Modelling the measurement

The dual-dye ratiometric photometric measurement procedures described in ISO 8655-7 and ISO 8655-8 use a cuvette containing a copper(II) chloride solution of known volume, which is determined by a gravimetric method. The POVA under test is used to add an unknown volume of test solution with known Ponceau S concentration to the cuvette containing $CuCl_2$ solution. The contents of the cuvette are mixed without removing the cuvette from the light path of the spectrophotometer, and absorbances at 520 nm and 730 nm are measured before and after the addition of test solution.

Calibrator solutions of $CuCl_2$ and Ponceau S are prepared, and their absorbance values at 520 nm and 730 nm measured. Preparation of the Ponceau S test solutions of different concentrations involves the preparation of dilutions, which are expressed by the dilution ratio, R. Absorbances of the calibrator solutions, together with the dilution ratio, R, are used to calculate the calibration constant, K, for a given concentration of Ponceau S.

The unknown volume of Ponceau S solution delivered by the POVA under test is calculated from the volume and absorbances of the $CuCl_2$ solution prior to addition of test solution, the calibration constant K, and the absorbance of the mixture in the cuvette after addition of test solution.

The Formula for the total volume $V_T(i)$ of delivered test solution after the i-th delivery at the test temperature is given by Formula (1):

$$V_T(i) = V_{C0} \frac{\dfrac{A_{M520}(i) - A_{C520}}{A_{C730} - A_{C520}}}{K_j - \dfrac{A_{M520}(i) - A_{C520}}{A_{C730} - A_{C520}}} \quad (1)$$

where

$V_T(i)$ is the total volume of test solution which has been added to the test cuvette from the first delivery through the i-th delivery;

V_{C0} is the actual volume of copper(II) chloride solution in the prepared test cuvette at the start of the test;

K_j is the calibration constant from Formula (2);

$A_{M520}(i)$ is the absorbance at 520 nm of the cuvette mixture after the i-th delivery of test solution;

A_{C520} is the absorbance at 520 nm of the copper(II) chloride solution in the cuvette prior to the first delivery of test solution;

A_{C730} is the absorbance at 730 nm of the copper(II) chloride solution in the cuvette prior to the first delivery of test solution.

The calibration constant (K_j) for each batch of solutions is calculated using Formula (2). Absorbance values are obtained from the measurements in ISO 8655-8:2022, 8.2.

$$K_j = \frac{1}{R_j}\left(\frac{A_{Cal520,j} - A_{CalC520}}{A_{CalC730} - A_{CalC520}}\right) \quad (2)$$

where

K_j is the calibration constant for the test-volume-specific calibrator solution [the subscript j refers to the test volume (V_S)];

R_j is the dilution ratio of the calibrator solution;

$A_{Cal520,j}$ is the absorbance of the Ponceau S calibrator solution j at 520 nm;

$A_{CalC520}$ is the absorbance of the $CuCl_2$ solution at 520 nm;

$A_{CalC730}$ is the absorbance of the $CuCl_2$ solution at 730 nm.

The dilution ratio (R) is calculated according to Formula (3).

$$R = \frac{V_{PS}}{V_{PS} + V_C} \quad (3)$$

where

R is the dilution ratio;

V_{PS} is the actual measured volume of Ponceau S solution;

V_C is the actual measured volume of copper(II) chloride solution.

Formulae (1), (2) and (3) contain nine input variables. Six of these inputs are photometric absorbance values. Three inputs are liquid volumes at the test temperature and each of these three volumes is determined by weighing on a balance.

The liquid volumes V_{C0}, V_{PS} and V_C at the test room temperature are calculated according to Formula (4).

$$V_L = (m_L - m_E) \times \frac{1}{\rho_L - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \tag{4}$$

where

V_L is the calculated volume at the temperature of the test liquid, in ml;

m_L is the balance indication of the weighing vessel after liquid delivery, in g;

m_E is the balance indication of the weighing vessel before liquid delivery, in g (m_E = 0 in case the balance was tared with the weighing vessel);

ρ_A is the density of air, in g/ml (see Formula (5) below);

ρ_B is the actual or assumed density of the weights used to calibrate the balance, in g/ml;

NOTE Stainless steel weights of density 8,0 g/ml are typically used for balance calibration.

ρ_L is the density of the liquid at the test temperature, in g/ml.

Formula (5) for the air density can be used at temperatures between 15 °C and 27 °C:

$$\rho_A = \frac{1}{1\ 000} \times \frac{0{,}348\ 48 \times P - 0{,}009 \times h_r \times e^{(0{,}061 \times t)}}{t + 273{,}15} \tag{5}$$

where

ρ_A is the air density, in g/ml;

t is the ambient temperature, in °C;

P is the barometric pressure, in hPa;

h_r is the relative air humidity, in %.

The relative uncertainty of Formula (5) is 2,4 × 10^{-4} g/ml under the following conditions: barometric pressure between 600 hPa and 1 100 hPa, ambient temperature between 15 °C and 27 °C, and relative humidity between 20 % and 80 %.

At other environmental conditions, Formula (5) is replaced with the CIPM-2007 calculations described in Reference [3].

According to ISO 8655-8, the mean volume is calculated according to Formula (6):

$$\bar{V} = \frac{V_T(n)}{n} \tag{6}$$

where

$\bar{V}$ is the mean volume;

$V_T(n)$ is the total volume of test solution in the cuvette after the n-th delivery (typically, n = 10).

If a cubic expansion coefficient γ for the POVA is known, it can be applied to correct the dispensed volume to the reference temperature using Formula (7).

$$V_{T,ref}(i) = V_T(i) \times [1 - \gamma(t_L - t_{ref})] \quad (7)$$

where

$V_{T,ref}(i)$ is the total volume of test liquid after the i-th delivery corrected to a reference temperature;

γ is the cubic thermal expansion coefficient for the POVA under test;

t_L is the temperature of the test liquid at the test room temperature;

t_{ref} is the reference temperature for the POVA, typically 20 °C or 27 °C.

5 General procedure for the uncertainty calculation

The evaluation of measurement uncertainty in this document follows ISO/IEC Guide 98-3. The method has the following steps:

a) Expressing, in mathematical terms, the relationship between the measurand and its input quantities.

b) Determining the expected value of each input quantity.

c) Determining the standard uncertainty of each input quantity.

d) Determining the degree of freedom for each input quantity.

e) Determining all covariance between the input quantities.

f) Calculating the expected value for the measurand.

g) Calculating the sensitivity coefficient of each input quantity.

h) Calculating the combined standard uncertainty of the measurand.

i) Calculating the effective degrees of freedom of the combined standard uncertainty.

j) Choosing an appropriate coverage factor, k, to achieve the required confidence level.

k) Calculating the expanded uncertainty.

In this document, the uncertainty of the measurement associated with the systematic error of the mean volume is separated into three different clauses: the uncertainty components associated with the photometric measuring system (Clause 6), the uncertainty components associated with the device under test (POVA, Clause 7) and the uncertainty components associated with the liquid delivery process (Clause 8).

6 Standard uncertainty components associated with the measuring system (photometric measurement procedure)

6.1 General information on the estimation of standard uncertainty components

It is possible to experimentally estimate the standard uncertainty of measurement, $u(x)$, for a quantity x, by performing repeated measurements of x under normal laboratory conditions. This is called a type A evaluation according to ISO/IEC Guide 98-3. The standard deviation of the obtained values is a measure of the repeatability of the measurement. The standard uncertainty associated with x can be the standard deviation (in the case where a single measurement of *x* is made), or the standard deviation of the mean equal to stdev(*x*)/sqrt(*n*) (in the case where *x* is the average of *n* readings).

See ISO/IEC Guide 98-3:2008,4.2 for more information on type A evaluation of standard uncertainty.

In addition to repeated measurements, the systematic component of the uncertainty of measurement for a quantity *x* is estimated by other means. This is called a type B evaluation according to ISO/IEC Guide 98-3. For example, one can obtain information for that estimation by considering the manufacturer's specifications of the POVA (e.g. resolution, linearity, drift, temperature dependence, etc.).

Often the manufacturer's specifications are given in the form of an interval covering the measurement value, with no additional information regarding distribution or coverage. In those cases, the measurement is assumed to follow a uniform or rectangular distribution. This distribution is characterized by a constant probability inside the interval while the probability outside the interval is zero.

The interval can be used to give the variance of *x* according to Formula (8):

$$u^2(x)=\frac{(a_+-a_-)^2}{12} \tag{8}$$

where

$u^2(x)$	is the variance of quantity x;
a_+ and a_-	give the upper and lower limits of the interval of the variable x.

The standard uncertainty, $u(x)$, is given as the square root of the variance.

In addition to uniform rectangular, other distributions are also possible when performing type B evaluations. See ISO/IEC Guide 98-3:2008, 4.3 for more information on type B evaluations of standard uncertainty.

6.2 Standard uncertainty of the copper(II) chloride solution volume

According to ISO 8655-7 and ISO 8655-8, the volume of copper(II) chloride solution (V_{C0}) is in the range of 4,5 ml to 5,5 ml and is within ±0,03 % of the chosen volume. For this example, the maximum specified error (±0,03 %) is modelled as a rectangular distribution, as shown in Formula (9).

$$u(V_{C0})=V_{C0}\times\frac{0{,}000\ 3}{\sqrt{3}} \tag{9}$$

where

$u(V_{C0})$	is the standard uncertainty associated with the volume of the copper(II) chloride solution;
V_{C0}	is the volume of copper(II) chloride solution in the cuvette.

EXAMPLE When V_{C0} is 5 000 µl, $u(V_{C0})$ is 0,866 0 µl with infinite degrees of freedom based on a rectangular distribution.

NOTE Formula (4) is used in the measurement of this volume (V_{C0}) at the temperature of the absorbance measurement. The uncertainty of the balances and other test equipment specified in ISO 8655-8 are sufficient to achieve a 3:1 measurement capability index versus this 0,03 % tolerance when using Formula (4).

6.3 Standard uncertainty of the cuvette mixture absorbance at 520 nm

The absorbance of the cuvette mixture at 520 nm after the *n*-th delivery, $A_{M520}(n)$, in a reference calibration (n = 10 or greater) is typically in the range of 0,50 to 1,2 absorbance units (AU). The uncertainty of this measurement is dominated by photometric repeatability of the spectrophotometer (0,01 % relative standard deviation or 0,000 05 AU, whichever is greater). There is also a contribution from the effect that allowable temperature change has on the chromophore (±0,5 °C rectangular). An example is given in Formula (10), where there is a relative uncertainty due to repeatability of 0,01 %, plus the 0,5 °C temperature limit (rectangular), multiplied by the dye sensitivity of 0,000 5 % per °C.

$$u[A_{M520}(n)] = A_{M520}(n) \times \sqrt{0{,}000\ 1^2 + 0{,}000\ 5^2 \times \frac{0{,}5^2}{3}} \qquad (10)$$

where

$u[A_{M520}(n)]$ is the standard uncertainty associated with the absorbance of the cuvette mixture at 520 nm;

$A_{M520}(n)$ is the absorbance of the cuvette mixture at 520 nm.

EXAMPLE For a 5 µl test volume and n = 10 replicates, $A_{M520}(n)$ is expected to be 0,681 7 AU and $u[A_{M520}(n)]$ is 1,197 × 10^{-4} AU with 285 degrees of freedom. This is based on an estimate of infinite degrees of freedom for the rectangular distribution of the temperature range, 30 degrees of freedom for the photometric repeatability, and applying the Welch-Satterthwaite formula in Clause 11.

6.4 Standard uncertainty of the cuvette starting absorbance at 730 nm

The starting absorbance of the cuvette at 730 nm before the first delivery (A_{C730}) is in the range of 1,0 AU to 1,3 AU. The uncertainty of this measurement is dominated by photometric repeatability of the spectrophotometer (0,01 % relative standard deviation) and a similar contribution from the effect that allowable temperature uncertainty (0,1 °C, k = 2) has on the $CuCl_2$ chromophore.

An example is given in Formula (11), where there is a relative uncertainty due to repeatability of 0,01 %, plus the 0,05 °C temperature uncertainty (k = 1), multiplied by the dye sensitivity of 0,001 65 % per °C.

$$u(A_{C730}) = A_{C730} \times \sqrt{0{,}000\ 1^2 + 0{,}001\ 65^2 \times 0{,}05^2} \qquad (11)$$

where

$u(A_{C730})$ is the standard uncertainty associated with the starting absorbance at 730 nm;

A_{C730} is the starting absorbance of the cuvette at 730 nm.

EXAMPLE When A_{C730} is 1,098 AU, then $u(A_{C730})$ is 1,423 × 10^{-4} AU with 58 degrees of freedom. This is based on an estimate of 30 degrees of freedom for the temperature uncertainty, 30 degrees of freedom for the photometric repeatability and applying the Welch-Satterthwaite formula in Clause 11.

6.5 Standard uncertainty of the cuvette starting absorbance at 520 nm

The starting absorbance of the cuvette at 520 nm before the first delivery (A_{C520}) is in the range of 0,015 AU to 0,025 AU. The uncertainty of this measurement is dominated by photometric repeatability specification of the spectrophotometer (0,000 05 AU standard deviation) and is shown in Formula (12). The contribution from temperature uncertainty is negligible.

$$u(A_{C520}) = 0{,}000\ 05 \qquad (12)$$

where $u(A_{C520})$ is the standard uncertainty associated with the starting absorbance at 520 nm.

EXAMPLE $u(A_{C520})$ is taken to be 5,000 × 10^{-5} AU with 30 degrees of freedom.

6.6 Standard uncertainty of the volume of Ponceau S and copper(II) choloride solutions used in calibrators

The dilution ratio, R, of the calibrators is calculated according to Formula (3).

In this example, each of these volumes (V_{PS} and V_C) is measured by weighing and calculated at the test temperature according to Formula (4). The typical values are in the range of several ml up to several litres, depending on calibrator solutions.

For V_{PS}, the relative standard uncertainty of weighing indication is estimated at 0,002 0 % (or better) of indicated value based on the minimum requirements for balances stated in ISO 8655-8. Two indications are required for each solution, empty and loaded, so this value appears twice in Formula (13) and twice in Formula (14).

The uncertainty in density also contributes to uncertainty in V_{PS}. The two primary contributors are the standard relative uncertainty (k = 1) of the density meter (0,002 5 %) and the effect that temperature uncertainty of the liquids has on the density of the Ponceau S solution, which is estimated as 0,001 05 % (based on 0,021 0 % per °C expansion, and 0,05 °C standard uncertainty in the liquid temperature). This uncertainty in V_{PS} is shown in Formula (13).

$$u(V_{PS})=V_{PS}\times\sqrt{0{,}000\ 02^2+0{,}000\ 02^2+0{,}000\ 025^2+0{,}000\ 010\ 5^2} \quad (13)$$

where

$u(V_{PS})$ is the standard uncertainty associated with the volume of Ponceau S solution used in the calibrator solutions;

V_{PS} is the volume of Ponceau S solution used in the calibrator solutions.

EXAMPLE 1 When V_{PS} is 5 ml, then $u(V_{PS})$ is 1,959 × 10^{-4} ml with 98 degrees of freedom. This is based on an estimate of 30 degrees of freedom for each of the four contributing uncertainties in Formula (13) and applying the Welch-Satterthwaite formula in Clause 11.

Similarly, the uncertainty in V_C is calculated as shown in Formula (14):

$$u(V_C)=V_C\times\sqrt{0{,}000\ 02^2+0{,}000\ 02^2+0{,}000\ 025^2+0{,}000\ 010\ 5^2} \quad (14)$$

where

$u(V_C)$ is the standard uncertainty associated with the volume of copper(II) chloride solution used in the calibrator solutions;

V_C is the volume of copper(II) chloride solution used in the calibrator solutions.

EXAMPLE 2 When V_C is 500 ml, then $u(V_C)$ is 1,959 × 10^{-2} ml with 98 degrees of freedom. This is based on an estimate of 30 degrees of freedom for each of the four contributing uncertainties in Formula (14) and applying the Welch-Satterthwaite formula in Clause 11.

From Formulae (13) and (14) it can be shown that there is a 0,003 9 % relative standard uncertainty in the measurements of V_{PS} and V_C.

6.7 Standard uncertainty of the absorbances of the calibrator solutions

The absorbances used to calculate the calibration constant K_j ($A_{Cal520,j}$, $A_{CalC520}$, and $A_{CalC730}$) are given in Formula (2) and described there. After the calibrator solutions are prepared, they are measured in the spectrophotometer.

The absorbance of the calibrator solution at 520 nm ($A_{Cal520,j}$) is typically in the range of 0,50 AU to 1,2 AU. The uncertainty of this measurement is dominated by photometric repeatability of the spectrophotometer (0,01 % relative standard deviation) with a lesser contribution from the effect that the temperature uncertainty has on the chromophore, i.e. 0,05 % per °C. The uncertainty in $A_{\mathrm{Cal520},j}$ is shown in Formula (15):

$$u(A_{\mathrm{Cal520},j}) = A_{\mathrm{Cal520},j} \times \sqrt{0{,}000\ 1^2 + (0{,}000\ 5 \times 0{,}05)^2} \tag{15}$$

where

$u(A_{\mathrm{Cal520},j})$ is the standard uncertainty associated with the absorbance at 520 nm of the calibrator solution *j*;

$A_{\mathrm{Cal520},j}$ is the absorbance at 520 nm of the calibrator solution *j*.

EXAMPLE 1 When $A_{\mathrm{Cal520},j}$ is 0,681 7 AU, then $u(A_{\mathrm{Cal520},j})$ is 7,027 × 10^{-5} AU with 34 degrees of freedom. This is based on an estimate of 30 degrees of freedom for the temperature uncertainty, 30 degrees of freedom for the photometric repeatability and applying the Welch-Satterthwaite formula in Clause 11.

The absorbance of the copper solution used to prepare the calibrator (A_{CalC730}) is measured at 730 nm and is in the range of 1,0 AU to 1,3 AU. The uncertainty of this measurement is dominated by photometric repeatability of the spectrophotometer (0,01 % relative standard deviation) and a contribution from the effect that the temperature uncertainty (0,05 °C, k = 1) has on the $CuCl_2$ chromophore, which has a temperature sensitivity of 0,165 % per degree. The uncertainty in A_{CalC730} is shown in Formula (16):

$$u(A_{\mathrm{CalC730}}) = A_{\mathrm{CalC730}} \times \sqrt{0{,}000\ 1^2 + (0{,}001\ 65 \times 0{,}05)^2} \tag{16}$$

where

$u(A_{\mathrm{CalC730}})$ is the standard uncertainty associated with the absorbance at 730 nm of the calibrator solution;

A_{CalC730} is the absorbance at 730 nm of the calibrator solution.

EXAMPLE 2 When A_{CalC730} is 1,098 0 AU, then $u(A_{\mathrm{CalC730}})$ is 1,423 × 10^{-4} AU with 58 degrees of freedom. This is based on an estimate of 30 degrees of freedom for the temperature uncertainty, 30 degrees of freedom for the photometric repeatability and applying the Welch-Satterthwaite formula in Clause 11.

The absorbance of the copper solution used to prepare the calibrator (A_{CalC520}) is also measured at 520 nm and is in the range of 0,015 AU to 0,025 AU. The uncertainty of this measurement is dominated by photometric repeatability specification of the spectrophotometer (0,000 05 AU standard deviation). Thermal contributions have a negligible effect on the uncertainty of this absorbance reading. The uncertainty in A_{CalC520} is shown in Formula (17):

$$u(A_{\mathrm{CalC520}}) = 0{,}000\ 05 \tag{17}$$

where $u(A_{\mathrm{CalC520}})$ is the standard uncertainty associated with the absorbance at 520 nm of the calibrator solution;

EXAMPLE 3 $u(A_{\mathrm{CalC520}})$ is taken to be 5,000 × 10^{-5} AU with 30 degrees of freedom.

7 Standard uncertainty components associated with the POVA

7.1 Standard uncertainty of the resolution

The standard uncertainty related to the resolution can be determined according to Formula (18):

$$u(res)=\frac{\Delta res}{\sqrt{12}} \tag{18}$$

where

$u(res)$ is the uncertainty associated with the resolution of the POVA's volume selection device;

Δres is the actual or estimated resolution of the volume selection device of the POVA.

NOTE The uncertainty related to the resolution of the POVA is included in the uncertainty budget when the measurements are dependent on the direct reading of the output volume, e.g. at a burette. The uncertainty of the resolution is also included when estimating the uncertainty to the systematic error e_S.

7.2 Standard uncertainty of the setting

The setting of the volume in the POVA is evaluated and included in the uncertainty budget, if applicable.

NOTE For example, the uncertainty related to the volume setting can be estimated using Formula (18).

7.3 Standard uncertainty related to air cushion effects

If applicable, the standard uncertainty related to the air cushion effect $u(\Delta V_{cush})$ depends on the size of the air cushion that is related to the lifting height in the pipette tip and can be calculated according to Formula (19), which is based on the information given in DKD-R 8-1:2011, Clause 8.7[4]:

$$u(\Delta V_{cush})=\sqrt{\left(u(V\Delta p)\times c_{V\Delta p}\right)^2+\left(u(V\Delta h_r)\times c_{V\Delta h_r}\right)^2+\left(u(V\Delta t_s)\times c_{V\Delta t_s}\right)^2} \tag{19}$$

where

$u(\Delta V_{cush})$ is the standard uncertainty related to the air cushion effect;

$u(V\Delta p)$ is the standard uncertainty attributed to air pressure variation during the tests;

$u(V\Delta h_r)$ is the standard uncertainty attributed to the humidity variation during the tests;

$u(V\Delta t_s)$ is the standard uncertainty caused by variation between the test liquid temperature, air temperature and temperature of the POVA under calibration;

c_i are the sensitivity coefficients related to each uncertainty component.

NOTE The variations of each parameter are determined experimentally during the test, and only apply to POVA which have an air cushion.

The sensitivity coefficients (c_i) related to the air cushion effect from pressure, humidity and pressure can be derived from DKD-R 8-1[4].

7.4 Standard uncertainty of the cubic expansion coefficient

The standard uncertainty related to the cubic expansion coefficient, γ, is dependent on knowledge of the actual material of the device under test and on the source of the data, which provides the user with an appropriate value. Data from the literature or manufacturer can be used for the expansion coefficient and this value would be expected to have a relative standard uncertainty of 5 % to 10 % of the expansion coefficient value for positive displacement devices, see Reference [5].

For devices with an air cushion, see 7.3, the thermal effects on the cubic expansion coefficient and the air cushion are entangled and are considered in tandem or determined experimentally. The details of this entanglement are beyond the scope of this document.

8 Standard uncertainty components associated with the liquid delivery process

8.1 Repeatability (experimental standard deviation)

Formulae (9) to (17) allow the determination of the standard uncertainties associated with the photometric measurement procedure. To derive the standard uncertainty associated with the liquid delivery process, the experimental standard deviation is included. When the mean delivered volume is the measurand, the standard deviation s_r is divided by the square root of the number of repeated measurements n as shown in Formula (20):

$$s_r(\bar{V}) = \frac{s_r}{\sqrt{n}} \tag{20}$$

where

$s_r(\bar{V})$ is the standard deviation of the mean delivered volume;

s_r is the repeatability standard deviation;

n is the number of replicate measurements.

NOTE To avoid uncertainty underestimation, the repeatability contribution $s_r(V_i) = s_r$ can be used instead of Formula (20).

8.2 Reproducibility

It is important to also include the uncertainty related to the reproducibility of $\bar{V}$ (from one test of the POVA to the next test), which is given by the symbol $s_d(\bar{V})$. There are several methods to determine this uncertainty contribution:

— a laboratory can perform experimental studies where the POVA test is repeatedly performed and the standard deviation of the measurement result $\bar{V}$ is calculated;

— A laboratory can refer to studies conducted and published by third parties, e.g. EURAMET or DKD;

— If no such information is available,

  — A value for reproducibility of 0,1 % of the selected volume can be used for pipettes (see References [6] and [7]). For other POVA instruments, different values are used. As no further information on the variation of individual measurements is taken into account, a rectangular distribution is suggested.

  — Alternatively, a standard uncertainty value for reproducibility as a fraction of the maximum permissible random error of the POVA can be used (see corresponding part of the ISO 8655 series). In this case, a normal distribution is suggested due to the underlying random nature of this limit value.

9 Combined standard uncertainty of measurement associated with the systematic error of mean volume

According to ISO/IEC Guide 98-3, when the errors of input quantities are uncorrelated, the variance characterizing the uncertainty of measurement is written according to Formula (21):

$$u^2 = \sum_i c_i^2 \times u^2(x_i) \quad (21)$$

where

u^2 is the variance characterizing the uncertainty of measurement;

$u^2(x_i)$ are the variances associated with each input quantity which contributes to the final result (described by the model);

c_i^2 are the squares of the sensitivity coefficients giving the degree of influence of each individual standard uncertainty.

The sensitivity coefficients are determined by evaluating the partial derivatives of the measurement formula by numerical simulations or by physical experiment. In the case of this document, it is possible to obtain explicit functions for many sensitivity coefficients by evaluating the partial derivatives as shown in Clause 10.

In this document, the uncertainty components are in groups corresponding to Clauses 6, 7, and 8. For the mean volume of a calibration or test, Formula (22) applies.

$$u^2\left(\bar{V}\right) = u_{\mathrm{MS}}^2\left(\bar{V}\right) + u_{\mathrm{POVA}}^2\left(\bar{V}\right) + u_{\mathrm{LDP}}^2\left(\bar{V}\right) \quad (22)$$

where

$u^2\left(\bar{V}\right)$ is the variance characterizing the uncertainty of the mean volume in a test or calibration;

$u_{\mathrm{MS}}^2\left(\bar{V}\right)$ is the variance characterizing the uncertainty due to the photometric measuring system;

$u_{\mathrm{POVA}}^2\left(\bar{V}\right)$ is the variance characterizing the uncertainty due to the POVA;

$u_{\mathrm{LDP}}^2\left(\bar{V}\right)$ is the variance characterizing the uncertainty due to the liquid delivery process.

For the photometric measurement procedures of ISO 8655-8 and ISO 8655-7:2022, Annex B, Formula (1) gives the total volume. For a calibration with n replicates, Formula (23) applies.

$$u_{\mathrm{MS}}^2\left(\bar{V}\right) = u^2\left[\frac{V_{\mathrm{T}}(n)}{n}\right] \quad (23)$$

where $V_{\mathrm{T}}(n)$ is the total dispensed volume in the cuvette after the n-th dispense.

The two liquid delivery process variances (Clause 8) can be combined as shown in Formula (24).

$$u_{\mathrm{LDP}}^2\left(\bar{V}\right) = \frac{s_r^2}{n} + s_d^2\left(\bar{V}\right) \quad (24)$$

where

$u_{\mathrm{LDP}}^2\left(\bar{V}\right)$ is the variance of the liquid delivery process;

s_r is the repeatability standard deviation;

$\frac{s_r^2}{n}$ is the variance of the mean volume due to the repeatability of the POVA;

$s_d^2(\overline{V})$ is the variance of the mean volume due to test process reproducibility.

10 Sensitivity coefficients

Sensitivities to the nine input variables described in Formulae (1), (2) and (3) are discussed in order of appearance, starting with sensitivity to V_{C0} (initial volume of $CuCl_2$ solution in the cuvette). Formula (6) and Formula (1) are combined, then differentiated with respect to V_{C0} resulting in Formula (25):

$$\frac{\partial \overline{V}}{\partial V_{C0}} = \frac{1}{n} \times \frac{V_T(n)}{V_{C0}} \tag{25}$$

The three absorbance values (A_{M520}, A_{C730}, and A_{C520}) which appear in Formula (1) are evaluated in a similar way and results are shown in Formulae (26), (27), and (28).

$$\frac{\partial \overline{V}}{\partial A_{M520}(n)} = \frac{1}{n} \times \frac{V_T(n)}{[A_{M520}(n) - A_{C520}]} \times \left[1 + \frac{V_T(n)}{V_{C0}}\right] \tag{26}$$

$$\frac{\partial \overline{V}}{\partial A_{C730}} = \frac{-1}{n} \times \frac{V_T(n)}{(A_{C730} - A_{C520})} \times \left[1 + \frac{V_T(n)}{V_{C0}}\right] \tag{27}$$

$$\frac{\partial \overline{V}}{\partial A_{C520}} = \frac{V_T(n)}{n} \times \left[1 + \frac{V_T(n)}{V_{C0}}\right] \times \frac{A_{M520}(n) - A_{C730}}{[A_{M520}(n) - A_{C520}](A_{C730} - A_{C520})} \tag{28}$$

Sensitivity of the mean volume to the calibration constant K_j appearing in Formula (1) is given in Formula (29):

$$\frac{\partial \overline{V}}{\partial K_j} = \frac{-V_T(n)}{n} \times \frac{V_T(n)}{V_{C0}} \times \frac{A_{C730} - A_{C520}}{A_{M520}(n) - A_{C520}} \tag{29}$$

The calibration constant K_j is given in Formula (2) and is sensitive to the dilution ratio, R_j, and the three absorbance values $A_{Cal520,j}$, $A_{CalC520}$, $A_{CalC730}$.

The sensitivity of K_j with respect to R_j is shown in Formula (30).

$$\frac{\partial K_j}{\partial R_j} = \frac{-1}{R_j^2} \times \left(\frac{A_{Cal520,j} - A_{CalC520}}{A_{CalC730} - A_{CalC520}}\right) \tag{30}$$

The dilution ratio, R_j, is given in Formula (3) which can be evaluated for sensitivity to the two input volumes V_{PS} and V_C as shown in Formulae (31) and (32).

$$\frac{\partial R_j}{\partial V_{PS}} = \frac{V_C}{(V_{PS} + V_C)^2} \tag{31}$$

$$\frac{\partial R_j}{\partial V_C} = \frac{-V_{PS}}{(V_{PS} + V_C)^2} \tag{32}$$

The sensitivity of K_j with respect to the absorbance values $A_{Cal520,j}$, $A_{CalC520}$, $A_{CalC730}$ is shown in Formulae (33), (34), and (35).

$$\frac{\partial K_j}{\partial A_{Cal520,j}} = \frac{1}{R_j} \times \left(\frac{1}{A_{CalC730} - A_{CalC520}} \right) \tag{33}$$

$$\frac{\partial K_j}{\partial A_{CalC730}} = \frac{-1}{R_j} \times \frac{A_{Cal520,j} - A_{CalC520}}{(A_{CalC730} - A_{CalC520})^2} \tag{34}$$

$$\frac{\partial K_j}{\partial A_{CalC520}} = \frac{1}{R_j} \times \frac{A_{Cal520,j} - A_{CalC730}}{(A_{CalC730} - A_{CalC520})^2} \tag{35}$$

Formula (29) can be combined with Formulae (30) and (31) as a multiplicative product to determine the sensitivity of the mean volume to V_{PS} as shown in Formula (36).

$$\frac{\partial \overline{V}}{\partial V_{PS}} = \frac{\partial \overline{V}}{\partial K_j} \times \frac{\partial K_j}{\partial R_j} \times \frac{\partial R_j}{\partial V_{PS}} \tag{36}$$

Similarly, the mean volume is sensitive to the volume of copper(II) chloride solution used in the dilution ratio in Formula (3). This sensitivity is shown in Formula (37):

$$\frac{\partial \overline{V}}{\partial V_C} = \frac{\partial \overline{V}}{\partial K_j} \times \frac{\partial K_j}{\partial R_j} \times \frac{\partial R_j}{\partial V_C} \tag{37}$$

The mean volume is sensitive to the three absorbance values $A_{Cal520,j}$, $A_{CalC520}$, $A_{CalC730}$ as shown in Formulae (38), (39), and (40).

$$\frac{\partial \overline{V}}{\partial A_{Cal520,j}} = \frac{\partial \overline{V}}{\partial K_j} \times \frac{\partial K_j}{\partial A_{Cal520,j}} \tag{38}$$

$$\frac{\partial \overline{V}}{\partial A_{CalC520}} = \frac{\partial \overline{V}}{\partial K_j} \times \frac{\partial K_j}{\partial A_{CalC520}} \tag{39}$$

$$\frac{\partial \overline{V}}{\partial A_{CalC730}} = \frac{\partial \overline{V}}{\partial K_j} \times \frac{\partial K_j}{\partial A_{CalC730}} \tag{40}$$

11 Coverage factor *k*

In order to calculate an appropriate coverage factor (k) for a 95 % confidence level (see ISO/IEC Guide 98-3:2008, Annex G) the effective degrees of freedom ν_{eff}, are estimated by means of the Welch-Satterthwaite formula as shown in Formula (41):

$$\nu_{eff} = \frac{u_V^4}{\sum_{i=1}^{n} \frac{u_i^4}{\nu_i}} \tag{41}$$

where

- ν_{eff} are the effective degrees of freedom for the measurement;
- u_V is the combined standard uncertainty of the measured volume;
- u_i is the standard uncertainty of each component;
- ν_i are the degrees of freedom of each component.

For 10 or more measurements, k can be calculated or k = 2 can be used if the individual standard uncertainty values have a similar statistical contribution to the combined uncertainty. For less than 10 measurements, k is calculated.

12 Expanded uncertainty of measurement associated with the volume $\bar{V}$

The expanded uncertainty of the volume, $\bar{V}$, is expressed according to Formula (42) where the standard uncertainty is multiplied by the coverage factor k.

$$U(\bar{V}) = k \times u(\bar{V}) \tag{42}$$

The result of the measurement can then be expressed with associated uncertainty according to Formula (43):

$$V_M = \bar{V} \pm U(\bar{V}) \tag{43}$$

where V_M is the overall result of the measurement including the expanded uncertainty of measurement.

13 Example for determining the uncertainty of the volume measurement of POVA

13.1 Measurement conditions

The measurement conditions are as follows:

— tenfold measurement of a selected volume of V_S = 5 µl of test solution (Ponceau S Solution No. 4, according to ISO 8655-8), delivered by an electronic syringe (per EURAMET Project No. 1486, reproducibility is 0,005 0 µl, see Reference [8]);

— photometer meeting the minimum requirements of ISO 8655-8:2022, Table 1;

— thermometer and other measuring instruments meeting the requirements of ISO 8655-8:2022, Table 2;

— mean volume: $\bar{V}$ = 5,014 µl ;

— random error of measurement (standard deviation, n = 10): s_r = 0,008 2 µl;

— standard deviation of the mean: $s_r(\bar{V}) = s_r / \sqrt{10}$ = 0,002 6 µl;

— systematic error of measurement: $e_S = \bar{V} - V_S$ = 0,014 µl.

The determination of the uncertainty for these conditions is given in Tables 1 and 2.

Table 1 — Measuring system standard uncertainty

Uncertainty component	Unit	Symbol	Estimation	Distribution	Standard uncertainty	Sensitivity coefficient	Uncertainty contribution µl	Degrees of freedom	Percent contribution %
Volume of $CuCl_2$ in cuvette	µl	V_{C0}	5 000	rectangular	$8,660 \times 10^{-1}$	$1,000 \times 10^{-3}$	$8,660 \times 10^{-4}$	∞	19
Absorbance at 520 nm of cuvette mixture at $n = 10$	AU	A_{M520}	0,681 7	combination (see 6.2)	$1,197 \times 10^{-4}$	7,609	$9,108 \times 10^{-4}$	285	21
Absorbance at 730 nm at $n = 0$	AU	A_{C730}	1,098 0	normal	$1,423 \times 10^{-4}$	−4,676	$-6,656 \times 10^{-4}$	58	11
Absorbance at 520 nm at $n = 0$	AU	A_{C520}	0,018 0	normal	$5,000 \times 10^{-5}$	−2,933	$-1,467 \times 10^{-4}$	30	0,5
Calibrator volume, Ponceau S solution	ml	V_{PS}	5	normal	$1,959 \times 10^{-4}$	1,000	$1,959 \times 10^{-4}$	98	1
Calibrator volume, $CuCl_2$ solution	ml	V_C	500	normal	$1,959 \times 10^{-2}$	−0,010	$-1,959 \times 10^{-4}$	98	1
Absorbance at 520 nm of calibrator solution j	AU	$A_{Cal520,j}$	0,681 7	normal	$7,027 \times 10^{-5}$	−7,609	$-5,347 \times 10^{-4}$	34	7
Absorbance at 730 nm of $CuCl_2$ calibrator solution	AU	$A_{CalC730}$	1,098 0	normal	$1,423 \times 10^{-4}$	4,676	$-6,656 \times 10^{-4}$	58	11
Absorbance at 520 nm of $CuCl_2$ calibrator solution	AU	$A_{CalC520}$	0,018 0	normal	$5,000 \times 10^{-5}$	2,933	$1,467 \times 10^{-4}$	30	0,5
Maximum evaporation of Ponceau S solution	AU	L_E	0,681 7	right triangular	$1,391 \times 10^{-4}$	7,609	$1,059 \times 10^{-3}$	∞	28
Measuring system standard uncertainty [a]	µl	u_{MS}					$1,998 \times 10^{-3}$	1 368	100

[a] The bottom row (measuring system standard uncertainty) is a summation of all other values in this table according to Formula (21).

Table 2 — Combined standard uncertainty of the mean volume

Uncertainty component	Unit	Symbol	Distribution	Standard uncertainty	Sensitivity coefficient	Uncertainty contribution µl	Degrees of freedom	Percent contribution %
Measuring system standard uncertainty [a]	µl	u_{MS}	normal	$1{,}998 \times 10^{-3}$	1	$1{,}998 \times 10^{-3}$	1 368	11
POVA standard uncertainty [b]	µl	u_{POVA}	rectangular	$2{,}887 \times 10^{-4}$	1	omitted [d]	infinite	0
Standard deviation of the mean [c]	µl	$s_r(\bar{V})$	normal	0,002 6	1	0,002 6	9	19
Reproducibility of the calibration (incl. operator) [c]	µl	$s_d(\bar{V})$	normal	0,005 0	1	0,005 0	50	70
Standard uncertainty of the calibration	µl	$u(\bar{V})$	normal	n/a	n/a	0,006 0	73	100

[a] Measuring system standard uncertainty is taken from Table 1.

[b] For the motorised syringe in this example, setting uncertainty (7.2) applies. Resolution (7.1), cubic expansion (7.4) and air cushion (7.3) do not apply to this example.

[c] Standard deviation of the mean and reproducibility of the calibration are described in Clause 8. These components describe the uncertainty of the liquid delivery process, u_{LDP} [see also Formula (24)]. Values for both are taken from Reference [8].

[d] Setting the syringe also contributes to reproducibility of the calibration and is included as part of the reproducibility data. It is omitted from the summation as an example of avoiding double counting. By examining the values in this table, it can be seen that u_{POVA} (display resolution when setting the syringe) is negligible compared to other sources of uncertainty.

13.2 Results

13.2.1 Standard uncertainty of the POVA mean volume

The standard uncertainty of the mean delivered volume is calculated according to Formula (44):

$$u(\bar{V}) = \sqrt{u_{MS}^2(\bar{V}) + u_{POVA}^2(\bar{V}) + u_{LDP}^2(\bar{V})} \quad (44)$$

$$u(\bar{V}) = 0{,}006\ 0\ \mu l$$

where

$u(\bar{V})$ is the standard uncertainty of the mean volume.

NOTE See Table 2 for the source of the 0,006 0 µl value.

13.2.2 Expanded uncertainty of the measurement

The expanded uncertainty of the measurement is calculated by multiplying the standard uncertainty of the measurement by the coverage factor *k*, according to Formula (42). In this example, a coverage factor of (*k* = 2) is used, but that value can vary (see Clause 11).

$$U\left(\overline{V}\right)=u\left(\overline{V}\right)\times k=0{,}012\ 0\ \mu\text{l}$$

where $U\left(\overline{V}\right)$ is the expanded uncertainty of the measurement.

NOTE If the coverage factor were calculated using the effective degrees of freedom from Table 2 (73 degrees of freedom), the value of *k* would be 2,01 by rounding down to 50 degrees of freedom and using the 95 % probability interval from ISO/IEC Guide 98-3:2008, Table G.2. The value of *k* would be 1,99 if calculated from the t-statistic using a 95 % probability interval and 73 degrees of freedom. In this example, the practice of using *k* = 2 for calibrations of *n* = 10 replicates is adopted.

13.2.3 Result of measurement

The overall result of the measurement including the expanded uncertainty of measurement can be expressed according to Formula (43).

$$V_M = 5{,}014\ \mu\text{l} \pm 0{,}012\ \mu\text{l}\ (k = 2).$$

13.2.4 Uncertainty in use and corrections for pressure changes

The uncertainty in use of a single delivered volume is discussed in Annex A. The volume correction due to changes in atmospheric pressure is discussed in Annex B.

NOTE Correction for atmospheric pressure does not apply to the positive displacement syringe used in this example.

13.2.5 General remarks

The numerical values of the quantity estimation, standard uncertainty, and sensitivity coefficients are volume dependent. It is not appropriate to use the values given in this example for other volumes.

For the example given in Table 2, the uncertainty associated with the standard deviation of the POVA and the reproducibility of the calibration comprise 19 % and 70 %, respectively, of the overall calibration uncertainty. This is typical of many POVA calibrations where the overall calibration uncertainty is dominated by the repeatability and reproducibility of the liquid delivery process, including operator effects.

13.2.6 Note on the conformity with ISO/IEC Guide 98-3

The term “random error of measurement” (as used in the ISO 8655 series) is equivalent to the term “experimental standard deviation” used in ISO/IEC Guide 98-3.

There is no direct equivalent to “systematic error of measurement” e_S (as used in the ISO 8655 series) that is found within ISO/IEC Guide 98-3.

NOTE 1 The term “instrumental bias” is found in ISO/IEC Guide 99[9] and is similar to “systematic error of measurement”, provided that the POVA is considered as a liquid measuring instrument and that care is taken regarding a positive or negative numerical sign in the result.

To evaluate the systematic error of measurement we can define a volume difference V_D as shown in Formula (45).

$$V_D = V_S - \bar{V} \quad (45)$$

where V_D is the difference between the selected volume V_S and the mean volume $\bar{V}$.

The uncertainty of this volume difference, V_D, includes the uncertainty associated with the mean volume, $\bar{V}$, and possibly an uncertainty associated with the resolution or setting of the selected volume, V_S (see 7.1 and 7.2). The uncertainty of the "volume difference" $u(V_D)$ and the uncertainty of the "systematic error of measurement" $u(e_S)$ are identical provided that the uncertainty intervals are symmetric, as is the case in the example of this document.

NOTE 2 The "systematic error of measurement" $e_S = \bar{V} - V_S$ used within the ISO 8655 series is reversed in sign compared to the volume difference V_D and the "instrumental bias" defined in the ISO/IEC Guide 99.

Annex A
(informative)

Approaches for the estimation of uncertainty in use of a single delivered volume

A.1 General

For POVA delivering volumes in the laboratory in daily use, the uncertainty in use of a single delivered volume can be estimated. This uncertainty is larger than the uncertainty of the mean delivered volume $u(\bar{V})$ described in 13.2 and differs in two major ways:

a) only a single delivered volume is considered, so that the experimental standard deviation s_r is used and not $s_r / \sqrt{n}$.

b) The systematic error of the POVA is considered, recognizing that many POVA users do not correct for systematic error in daily use.

The approaches in this annex are practical simplifications and are not covered in the uncertainty concept of ISO/IEC Guide 98-3. It is good practice to determine whether these approaches cover a laboratory's specific application.

Numerical values used in the sample calculations in A.2 and A.3 are taken from 13.1 for purposes of illustration. When applying this concept, calculations are based on numerical values from data acquired in the laboratory where the POVA is used.

A.2 Uncertainty of a single delivered volume from a test data set

As an intermediate step, the uncertainty of a single delivered volume can be determined from a test data set of the POVA, e.g. from the data provided on a calibration certificate or test report associated with calibration, testing or routine tests. The uncertainty of a single delivered volume from a test data set applies to the conditions during the test event. Formula (A.1) describe the calculation of the uncertainty of a single delivered volume from a test data set:

$$u_{\text{sd,test}}(V) = \sqrt{u_{\text{MS}}^2 + s_r^2} \qquad \text{(A.1)}$$

$$u_{\text{sd,test}}(V) = \sqrt{(0{,}002\ 0)^2 + (0{,}008\ 2)^2}\ \mu\text{l} = 0{,}008\ 4\ \mu\text{l}$$

where

$u_{\text{sd,test}}(V)$	is the standard uncertainty of a single delivered volume during a test, in µl;
u_{MS}	is the standard uncertainty of the measuring system, in µl;
s_r	is the random error, calculated as standard deviation of the test data set, in µl.

In this example, the same value for uncertainty of the photometric measurement procedure is used as in Table 1. However, s_r is used instead of $s_r / \sqrt{10}$, because only a single delivered volume is considered.