TECHNICAL REPORT

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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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# 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).

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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):

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

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);

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

$\rho_B$ 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.

$\rho_L$ 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

$\rho_A$ 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 $\gamma$ 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;

$\gamma$ 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.

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

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.

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

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.

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

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.