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**Piston-operated volumetric  
instruments — Determination of  
uncertainty for volume measurements  
made using the photometric method**

*Instruments volumétriques actionnés par piston — Détermination de  
l'incertitude de mesure pour les mesurages volumétriques au moyen de  
la méthode photométrique*

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## Foreword

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# Piston-operated volumetric instruments — Determination of uncertainty for volume measurements made using the photometric method

## 1 Scope

This Technical Report gives the detailed evaluation of uncertainty for volume measurements according to the *Guide to the expression of uncertainty in measurements* (GUM). ISO 8655-7 covers non-gravimetric methods of volume measurement. ISO 8655-7:—, Annex A, presents the details of a method that employs removable cells (vials) in a UV/VIS photometer. ISO 8655-7:—, Annex B, presents the details of a method that employs a flow cell fixed in place in a UV/VIS photometer. This Technical Report covers the uncertainty of measurement of both methods.

## 2 Uncertainty analysis for the replaceable cell photometric method as described in ISO 8655-7:—, Annex A

### 2.1 Uncertainties of each measurand

For purposes of creating an uncertainty budget, the uncertainty for each device used in the determination of unknown volume has been taken from manufacturer's literature. The uncertainty for the system linearity was measured using a reference UV/VIS photometer. UV/VIS photometric uncertainties are based on ISO 8655-7:—, Table A.1.

Table 1 — Uncertainties of the analytical devices used

Item	Laboratory grade UV/VIS-photometer	Reference grade UV/VIS-photometer	Type of uncertainty
Flask, Class A volumetric, 1 000 ml	0,4 ml		B
Pipette, Class A volumetric, 100 ml	0,08 ml		B
Pipette, Class A volumetric, 10 ml	0,02 ml		B
Pipette, Class A volumetric, 5 ml	0,015 ml		B
Pipette, Class A volumetric, 2 ml	0,010 ml		B
Photometric measurement at $A = 0$	0,001 0	0,000 3	A
Photometric measurement at $A = 0,5$	0,001 5	0,000 5	A
Photometric measurement at $A = 1,0$	0,001 5	0,000 5	A
Photometric measurement at $A = 1,5$	0,002 0	0,000 7	A
Temperature of sample	0,2 °C		B
Reproducibility of UV/VIS photometer wavelength	0,5 nm	0,2 nm	B
Non-linearity of photometric response with 2:1 dynamic range	—	0,14 %	A
Non-linearity of photometric response with 8:1 dynamic range	—	0,63 %	A

**2.2 Mathematical model of method**

The unknown volume is calculated based on the Beer-Lambert Law. The stock solution, if Ponceau S is used as chromophore, has extinction coefficient  $\epsilon_1$  at 520 nm, and zero absorbance at 730 nm. The concentration of the stock solution is  $C_S$ . The diluent has extinction coefficient  $\epsilon_2$  at its peak wavelength 730 nm and concentration  $C_D$ .

A standard is created by mixing stock and diluent very accurately using large volumes. An empty vial is placed in the cell holder; it is filled with buffer and the UV/VIS photometer is set to zero at both wavelengths. The buffer is removed without disturbing the vial and it is rinsed and filled with the standard solution. The absorbance of the standard at the two wavelengths, 520 nm and 730 nm respectively is:

$$A_{S1} = \epsilon_1 C_S R l_{SV} \tag{1}$$

$$A_{S2} = \epsilon_2 C_D (1 - R) l_{SV} \tag{2}$$

where  $R$  is the dilution ratio of stock to diluent used to create the standard, and  $l_{SV}$  is the path length of the vial containing the aliquot of standard solution.

Next, a fresh vial containing a measured amount  $V_D$  of diluent is put into the UV/VIS photometer and a reading is taken at both wavelengths. The reading at 520 nm,  $A_{D1}$ , serves the purpose of establishing zero, which may vary from that of a vial containing buffer because of vial imperfections. This reading at 520 nm is subtracted from the reading at 730 nm,  $A_{D2}$ , to make the zero correction:

$$\text{Absorbance of diluent} = A_{D2} - A_{D1} = \epsilon_2 C_D l_S \tag{3}$$

The path length of this vial is  $l_S$ . Now the device under test is used to deliver an aliquot of stock solution into the diluent. The contents are mixed, and the absorbance is measured at 520 nm. The corrected absorbance of the unknown mixture at 520 nm is:

$$\text{Absorbance of unknown} = A_U - A_{D1} = \epsilon_1 C_S \left( \frac{V_U}{V_U + V_D} \right) l_S \tag{4}$$

By dividing the last two equations, one obtains

$$\frac{A_U - A_{D1}}{A_{D2} - A_{D1}} = \left( \frac{\epsilon_1 C_S}{\epsilon_2 C_D} \right) \left( \frac{V_U}{V_U + V_D} \right) \tag{5}$$

The two equations from the standard can likewise be divided to give:

$$\frac{A_{S1}}{A_{S2}} = \left( \frac{\epsilon_1 C_S}{\epsilon_2 C_D} \right) \left( \frac{R}{1 - R} \right) \tag{6}$$

Between these two equations the extinction coefficients and concentrations can be eliminated, giving:

$$\frac{A_U - A_{D1}}{A_{D2} - A_{D1}} = \left( \frac{1 - R}{R} \right) \left( \frac{V_U}{V_U + V_D} \right) \frac{A_{S1}}{A_{S2}} \tag{7}$$

All of these quantities have been measured except for  $V_U$ . This equation can be solved for  $V_U$  with the following result:

$$V_U = V_D \left[ \frac{\frac{A_U - A_{D1}}{A_{D2} - A_{D1}}}{\left(\frac{1-R}{R}\right) \frac{A_{S1}}{A_{S2}} - \left(\frac{A_U - A_{D1}}{A_{D2} - A_{D1}}\right)} \right] \quad (8)$$

This equation allows calculation of  $V_U$  based on measured quantities. Note that the path lengths of the vials do not enter into the result, even though two vials were used having different path lengths. Also, any variation in zero due to imperfections in the vials has been fully corrected. Measured absorbances appear as ratios, meaning that the UV/VIS photometer need not be accurate, only linear. No information is needed about extinction coefficients or concentrations of stock or diluent solutions.

### 2.3 Sensitivity coefficients

Sensitivity coefficients are obtained by taking partial derivatives of Equation (8) with respect to each of the measurands or other system parameters that can vary in a way to cause uncertainty in the results.

For the purpose of easily obtaining these partial derivatives, Equation (8) can be simplified by using the following approximations:

$$R \ll 1 \quad (9)$$

$$\frac{A_U - A_{D1}}{A_{D2} - A_{D1}} < 1 \quad (10)$$

$$A_{D1} \approx 0 \quad (11)$$

Concentrations of the stock solutions, dilution ratios and diluent have been created such as to make these approximations valid. With these approximations, Equation (8) becomes

$$V_U \approx V_D \frac{A_{S2} R}{A_{S1}} \left( \frac{A_U - A_{D1}}{A_{D2} - A_{D1}} \right) \quad (12)$$

This simplification creates an error no greater than 4 % in the sensitivity coefficients. They are in any case estimates, and this error is small compared to other possible sources of error. For purposes of illustrating the method of analysis, the values given in Table 2, which pertain to the calibration of a 0,5 µl pipette, are used.

**Table 2 — Values for calibration of a 0,5 µl pipette using the replaceable cell method**

$l = 1,8 \text{ cm}$	path length of vial used in making the photometric reading	
$V_S = 5 \text{ ml}$	volume of stock used to make the first dilution for the standard	
$V_{d1} = 1\ 000 \text{ ml}$	volume of diluent used to make the first dilution for the standard	
$V_{m1} = 5 \text{ ml}$	volume of the first mixture (dilution) used to make the second dilution	
$V_{d2} = 100 \text{ ml}$	volume of diluent used to make the second dilution	
$R = \left(\frac{5}{1005}\right)\left(\frac{5}{105}\right) = 2,369 \times 10^{-4}$	dilution ratio of standard	
$A_{SK} = 1\ 111 \text{ abs/cm}$	absorbance per unit path length of the undiluted stock solution at $\lambda_1$	
$A_D = 0,600\ 0 \text{ abs/cm}$	absorbance per unit path length of the diluent at $\lambda_2$	
$A_{S1} = (111\ 1)(1,8)(2,369 \times 10^{-4}) = 0,473\ 8 \text{ abs}$	absorbance of standard at $\lambda_1$	
$A_{S2} = (1 - 2,369 \times 10^{-4})(0,600)(1,8) = 1,079\ 7 \text{ abs}$	absorbance of standard at $\lambda_2$	
$A_{D1} = 0$	absorbance of diluent at $\lambda_1$	
$A_{D2} = (0,600 \text{ abs/cm})(1,8 \text{ cm}) = 1,080 \text{ abs}$	absorbance of diluent at $\lambda_2$	
$V_D = 5\ 000 \text{ µl}$	volume of diluent used to dilute the unknown sample	
$A_U = (1\ 111 \text{ abs/cm})(1,8 \text{ cm}) \left(\frac{0,5}{5\ 000,5}\right) = 0,200 \text{ abs}$	absorbance of unknown at $\lambda_1$	
$V_U = 0,500 \text{ µl}$	ISO/TR 16153:2004	

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In this example, the standard is produced by first diluting 5 ml of stock solution with 1 000 ml of diluent, and then diluting 5 ml of that with 100 ml of diluent.

**2.3.1 Uncertainty in reading of standard due to instrument imprecision at wavelength  $\lambda_1$**

In all of the following examples, the formulae are followed by a numeric evaluation using the parameters given above for a 0,5 µl pipette.

The partial derivative of Equation (12) with respect to  $A_{S1}$  is

$$\frac{\partial V_U}{\partial A_{S1}} = - \frac{V_D A_{S2} R}{A_{S1}^2} \left( \frac{A_U - A_{D1}}{A_{D2} - A_{D1}} \right) = - 1,055 \text{ µl/abs} \tag{13}$$

The sign is not relevant, since all sensitivity coefficients are squared to get the weighted variance.

The standard uncertainty in absorbance  $A_{S1}$  arises from two sources: the measurement at zero absorbance, which has an uncertainty of 0,000 3, and the measurement at an absorbance of 0,5 which has an uncertainty of 0,000 5. They combine as the square root of the sum of the squares:

$$U(A_{S1}) = \sqrt{0,000\ 3^2 + 0,000\ 5^2} \text{ abs} = 0,000\ 583 \text{ abs} \tag{14}$$



The standard uncertainty in volume measurement due to this uncertainty in  $A_{S1}$  is the product of the uncertainty in absorbance and the sensitivity factor:

$$U(V_{0,5})_{A_{S1}} = U(A_{S1}) \frac{\partial V_U}{\partial A_{S1}} = (0,000\ 583\ \text{abs})(1,055\ \mu\text{l/abs}) = 0,000\ 615\ \mu\text{l} = 6,15 \times 10^{-4}\ \mu\text{l} \quad (15)$$

### 2.3.2 Uncertainty in reading of standard due to instrument imprecision at wavelength $\lambda_2$

$$\begin{aligned} \frac{\partial V_U}{\partial A_{S2}} &= \frac{V_D R}{A_{S1}} \left( \frac{A_U - A_{D1}}{A_{D2} - A_{D1}} \right) \\ &= 0,463\ \mu\text{l/abs} \end{aligned} \quad (16)$$

The standard uncertainty in absorbance  $A_{S2}$  arises from two sources: the measurement at zero absorbance, which has an uncertainty of 0,000 3, and the measurement at 1,08 absorbance which has an uncertainty of 0,000 5. They combine as the square root of the sum of the squares:

$$U(A_{S1}) = \sqrt{0,000\ 3^2 + 0,000\ 5^2}\ \text{abs} = 0,000\ 583\ \text{abs} \quad (17)$$

The standard uncertainty in volume measurement due to this uncertainty in  $A_{S2}$  is the product of the uncertainty in absorbance and the sensitivity factor:

$$U(V_{0,5})_{A_{S2}} = U(A_{S2}) \frac{\partial V_U}{\partial A_{S2}} = (0,000\ 583\ \text{abs})(0,463\ \mu\text{l/abs}) = 2,70 \times 10^{-4}\ \mu\text{l} \quad (18)$$

### 2.3.3 Uncertainty in reading absorbance $A_U$ due to instrument imprecision at $\lambda_1$ (520 nm)

The partial derivative of Equation (12) with respect to  $A_U$  is 4

$$\begin{aligned} \frac{\partial V_U}{\partial A_U} &= V_D \frac{A_{S2} R}{A_{S1}(A_{D2} - A_{D1})} \\ &= 2,50\ \mu\text{l/abs} \end{aligned} \quad (19)$$

The standard uncertainty in absorbance  $A_U$  at 0,2 absorbance units has an uncertainty of 0,000 5 (in this case the specification for the spectrophotometer does not include a specification at 0,2, so the assumption is made that the uncertainty is that specified at the next greatest absorbance). The measurement at zero absorbance is covered in 2.3.4. Thus,

$$U(A_U) = 0,000\ 5\ \text{abs} \quad (20)$$

The standard uncertainty in volume measurement due to this uncertainty in  $A_U$  is the product of the uncertainty in absorbance and the sensitivity factor:

$$U(V_{0,5})_{A_U} = U(A_U) \frac{\partial V_U}{\partial A_U} = (0,000\ 5\ \text{abs})(2,50\ \mu\text{l/abs}) = 1,25 \times 10^{-3}\ \mu\text{l} \quad (21)$$

### 2.3.4 Uncertainty in reading diluent absorbance $A_{D1}$ due to instrument imprecision at $\lambda_1$ (520 nm)

$$\begin{aligned} \frac{\partial V_U}{\partial A_{D1}} &= \left( \frac{V_D A_{S2} R}{A_{S1}} \right) \left[ \left( \frac{-1}{A_{D2} - A_{D1}} \right) + \left( \frac{A_U - A_{D1}}{(A_{D2} - A_{D1})^2} \right) \right] \\ &= 2,04\ \mu\text{l/abs} \end{aligned} \quad (22)$$

The absorbance of the diluent at 520 nm is nearly zero, so the imprecision in the reading of  $A_{D1}$  is 0,000 3. This is the only source of uncertainty in this initial determination of absorbance. It is used in two places, to provide a zero for the absorbance of the unknown  $A_U$  and the absorbance of the diluent at 730 nm,  $A_{D1}$ .

$$U(A_{D1}) = 0,000\ 3\ \text{abs} \quad (23)$$

The uncertainty in volume measurement due to this is:

$$U(V_{0,5})_{A_{D1}} = U(A_{D1}) \frac{\partial V_U}{\partial A_{D1}} = (0,000\ 3\ \text{abs})(2,04\ \mu\text{l/abs}) = 6,12 \times 10^{-4}\ \mu\text{l} \quad (24)$$

**2.3.5 Uncertainty in reading diluent absorbance  $A_{D2}$  due to instrument imprecision at  $\lambda_2$  (730 nm)**

$$\begin{aligned} \frac{\partial V_U}{\partial A_{D2}} &= \left( \frac{V_D A_{S2} R}{A_{S1}} \right) \left\{ - \left[ \frac{A_U - A_{D1}}{(A_{D2} - A_{D1})^2} \right] \right\} \\ &= 0,463\ \mu\text{l/abs} \end{aligned} \quad (25)$$

The absorbance of the diluent measured at 730 nm is about 1, so

$$U(A_{D2}) = 0,000\ 5\ \text{abs} \quad (26)$$

The uncertainty in  $V_U$  due to this is:

$$U(V_{0,5})_{A_{D2}} = U(A_{D2}) \frac{\partial V_U}{\partial A_{D2}} = (0,000\ 5\ \text{abs})(0,463\ \mu\text{l/abs}) = 2,32 \times 10^{-4}\ \mu\text{l} \quad (27)$$

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**2.3.6 Uncertainty in readings due to drift in wavelength  $\lambda_1$  of photometer between the time that the standard is read and the unknown is read**

The magnitude of the uncertainty depends on the slope of the absorbance versus wavelength curve at the wavelength chosen for making the readings. For the chromophore chosen in this case at 520 nm, the slope is no greater than 0,1 % nm<sup>-1</sup>. The reading of the standard is taken as the correct wavelength, and it is the change from that which creates the uncertainty.

$$\begin{aligned} \frac{\partial V_U}{\partial \lambda_1} &= \left( \frac{\partial V_U}{\partial A_U} \right) \left( \frac{\partial A_U}{\partial \lambda_1} \right) \\ &= \left( \frac{V_D A_{S2} R}{A_{S1} A_{D2}} \right) (0,001)\ \mu\text{l/nm} \\ &= 2,50 \times 10^{-3}\ \mu\text{l/nm} \end{aligned} \quad (28)$$

The specification of the photometer is for a drift of no more than 0,2 nm. A rectangular probability is assumed, giving an uncertainty of:

$$U(\lambda_1) = 0,2\ \text{nm} / \sqrt{3} = 0,115\ \text{nm} \quad (29)$$

The square root of three is the conversion from a rectangular to standard uncertainty, as specified by the GUM. The resulting uncertainty in volume measurement due to uncertainty in wavelength is:

$$U(V_{0,5})_{\lambda_1} = U(\lambda_1) \frac{\partial V_U}{\partial \lambda_1} = (0,115\ \text{nm})(0,002\ 5\ \mu\text{l/nm}) = 2,89 \times 10^{-4}\ \mu\text{l} \quad (30)$$

### 2.3.7 Uncertainty in readings due to drift in wavelength $\lambda_2$ of photometer between the time that the standard is read and the unknown is read

The magnitude of the uncertainty depends on the slope of the absorbance versus wavelength curve at 730 nm for the chromophore in the diluent, in this case no greater than 0,05 % nm<sup>-1</sup>. The reading of the standard is taken as the correct wavelength, and it is the change from that which creates the uncertainty.

$$\begin{aligned}\frac{\partial V_U}{\partial \lambda_2} &= \left( \frac{\partial V_U}{\partial A_{D2}} \right) \left( \frac{\partial A_{D2}}{\partial \lambda_2} \right) \\ &= \left( -\frac{V_D A_{S2} R A_U}{A_{S1} A_{D2}^2} \right) (0,000 5) \mu\text{l/nm} \\ &= 2,31 \times 10^{-4} \mu\text{l/nm}\end{aligned}\quad (31)$$

The specification of the spectrophotometer is for a drift of no more than 0,2 nm. A rectangular probability is assumed, giving an uncertainty of:

$$U(\lambda_2) = 0,2 \text{ nm} / \sqrt{3} = 0,115 \text{ nm} \quad (32)$$

The resulting uncertainty in volume measurement due to uncertainty in wavelength is:

$$U(V_{0,5})_{\lambda_2} = U(\lambda_2) \frac{\partial V_U}{\partial \lambda_2} = (0,115 \text{ nm})(0,000 23 \mu\text{l/nm}) = 2,67 \times 10^{-5} \mu\text{l} \quad (33)$$

### 2.3.8 Uncertainty in the volume of stock used in creating the standard

In this case there was a double dilution performed in making the standard. We first address the uncertainty in  $V_U$  due to uncertainty in the volume of stock used in creating the first dilution. An uncertainty in volume of stock results in an uncertainty in the ratio of dilution for the standard, which in turn results in an uncertainty in the determination of unknown volume. In the following analysis,  $R_1$  is the ratio of dilution in the first mixture, and  $R_2$  is the dilution of that mixture to create the final standard.

$V_{d1}$  is the volume of diluent used in preparing the first dilution.

$$R = R_1 R_2 \quad (34)$$

$$R_1 = \frac{V_S}{V_{d1} + V_S} \approx \frac{V_S}{V_{d1}} \quad (35)$$

$$\frac{\partial R}{\partial V_S} = R_2 \frac{\partial R_1}{\partial V_S} = R_2 \frac{1}{V_{d1}} \quad (36)$$

$$\begin{aligned}\frac{\partial V_U}{\partial V_S} &= \frac{\partial V_U}{\partial R} \frac{\partial R}{\partial V_S} \\ &= \left( V_D \frac{A_{S2}}{A_{S1}} \frac{A_U}{A_{D2}} \right) \left( \frac{R_2}{V_{d1}} \right) \\ &= 1,00 \times 10^{-4} \mu\text{l}/\mu\text{l}\end{aligned}\quad (37)$$

The uncertainty in stock volume  $V_S$  when delivered by a 5 ml Class A pipette made from glass is found by dividing its tolerance of 0,015 ml by the factor  $\sqrt{3}$  to convert from a rectangular probability distribution to a normal one:

$$U(V_S) = 0,015 \text{ ml} / \sqrt{3} = 8,66 \mu\text{l} \quad (38)$$

The resulting uncertainty in unknown volume  $V_U$  due to uncertainty in stock volume is:

$$U(V_{0,5})_{V_S} = U(V_S) \frac{\partial V_U}{\partial V_S} = (8,66 \mu\text{l})(0,000 100 \mu\text{l}/\mu\text{l}) = 8,66 \times 10^{-4} \mu\text{l} \quad (39)$$

**2.3.9 Uncertainty in the volume of mixture 1 ( $V_{m1}$ , result of dilution 1) used in creating the second dilution**

The analysis follows that given in 2.3.8.

The volume of diluent used in preparing the second dilution is  $V_{d2}$ .

$$R = R_1 R_2 \quad (40)$$

$$R_2 = \frac{V_{m1}}{V_{d2} + V_{m1}} \approx \frac{V_{m1}}{V_{d2}} \quad (41)$$

$$\frac{\partial R}{\partial V_{m1}} = R_1 \frac{\partial R_2}{\partial V_{m1}} = R_1 \frac{1}{V_{d2}} \quad (42)$$

$$\begin{aligned} \frac{\partial V_U}{\partial V_{m1}} &= \frac{\partial V_U}{\partial R} \frac{\partial R}{\partial V_{m1}} \\ &= \left( V_D \frac{A_{S2}}{A_{S1}} \frac{A_U}{A_{D2}} \right) \left( \frac{R_1}{V_{d2}} \right) \\ &= 1,05 \times 10^{-4} \mu\text{l}/\mu\text{l} \end{aligned} \quad (43)$$

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A portion of the first mixture  $V_{m1}$  is measured and dispensed using a 5 ml Class A pipette. The uncertainty in this volume is

$$U(V_{m1}) = 0,015 \text{ ml} / \sqrt{3} = 8,66 \mu\text{l} \quad (44)$$

The resulting uncertainty in unknown volume  $V_U$  due to uncertainty in the volume of mixture 1 is:

$$U(V_{0,5})_{V_{m1}} = U(V_{m1}) \frac{\partial V_U}{\partial V_{m1}} = (8,66 \mu\text{l})(0,000 1 \mu\text{l}/\mu\text{l}) = 9,09 \times 10^{-4} \mu\text{l} \quad (45)$$

**2.3.10 Uncertainty in volume of diluent  $V_{d1}$  used in creating the first dilution**

$$\frac{\partial V_U}{\partial V_{d1}} = \frac{\partial V_U}{\partial R} \frac{\partial R}{\partial V_{d1}} \quad (46)$$

$$R = R_1 R_2 \quad (47)$$

$$\frac{\partial R}{\partial V_{d1}} = R_2 \frac{\partial R_1}{\partial V_{d1}} \quad (48)$$

$$R_1 = \frac{V_S}{V_S + V_{d1}} \approx \frac{V_S}{V_{d1}} \quad (49)$$