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**Piston-operated volumetric apparatus —  
Part 7:  
Non-gravimetric methods for the  
assessment of equipment performance**

*Appareils volumétriques à piston —*

*Partie 7: Méthodes non gravimétriques pour l'estimation de la  
performance d'équipement*

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# Contents

Page

Foreword.....	iv
Introduction .....	v
<b>1</b> <b>Scope</b> .....	<b>1</b>
<b>2</b> <b>Normative references</b> .....	<b>1</b>
<b>3</b> <b>Terms and definitions</b> .....	<b>2</b>
<b>4</b> <b>Principle</b> .....	<b>2</b>
<b>4.1</b> <b>Photometric method</b> .....	<b>2</b>
<b>4.2</b> <b>Titrimetric method</b> .....	<b>2</b>
<b>5</b> <b>Reagents</b> .....	<b>3</b>
<b>5.1</b> <b>Reagents for photometric method</b> .....	<b>3</b>
<b>5.2</b> <b>Reagents for titrimetric method</b> .....	<b>3</b>
<b>6</b> <b>Apparatus</b> .....	<b>4</b>
<b>6.1</b> <b>Photometric method</b> .....	<b>4</b>
<b>6.2</b> <b>Titrimetric method</b> .....	<b>4</b>
<b>7</b> <b>Test conditions</b> .....	<b>5</b>
<b>8</b> <b>Procedure</b> .....	<b>5</b>
<b>8.1</b> <b>General</b> .....	<b>5</b>
<b>8.2</b> <b>Photometric method</b> .....	<b>5</b>
<b>8.3</b> <b>Titrimetric method</b> .....	<b>5</b>
<b>9</b> <b>Calculation</b> .....	<b>6</b>
<b>10</b> <b>Test report</b> .....	<b>6</b>
<b>Annex A</b> (informative) <b>Example 1 for photometric test method</b> .....	<b>7</b>
<b>Annex B</b> (informative) <b>Example 2 for photometric test method</b> .....	<b>13</b>
<b>Annex C</b> (informative) <b>Example for titration test method</b> .....	<b>17</b>
<b>Bibliography</b> .....	<b>21</b>

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 8655-7 was prepared by Technical Committee ISO/TC 48, *Laboratory glassware and related apparatus*, Subcommittee SC 6, *Laboratory and volumetric ware*.

ISO 8655 consists of the following parts, under the general title *Piston-operated volumetric apparatus*:

- *Part 1: Terminology, general requirements and user recommendations*
- *Part 2: Piston pipettes*
- *Part 3: Piston burettes*
- *Part 4: Dilutors*
- *Part 5: Dispensers*
- *Part 6: Gravimetric methods for the determination of measurement error*
- *Part 7: Non-gravimetric methods for the assessment of equipment performance*

## Introduction

The ISO 8655 series was developed in order to specify the differing types of piston-operated volumetric apparatus and to provide a reference method and alternative test methods for verifying their characteristics covering the volume range typically from:

- the smallest hand-held pipetting devices, e.g. 1  $\mu\text{l}$ , up to
- the largest laboratory bench-standing volume dispensing instruments, e.g. 100 ml.

ISO 8655-1 provides general requirements and terminology. The detailed volumetric ranges for each type of apparatus specified in the ISO 8655 series are indicated in the appropriate tables of maximum permissible error, i.e. for piston pipettes (ISO 8655-2), for piston burettes (ISO 8655-3), for dilutors (ISO 8655-4) and for dispensers (ISO 8655-5).

ISO 8655-6 is the reference method for type testing and conformity testing. It is gravimetric and contains precise instructions designed to limit variation in procedure and thereby the potential for sources of error – a necessity for type and conformity testing.

The photometric and titrimetric methods described in this part of ISO 8655, are deliberately given as outline methods (see examples in the informative annexes), so that individual laboratories having their own equipment available, and working to different uncertainty requirements, may adapt either these methods, or the gravimetric method, accordingly. If the laboratories operate under ISO 9000 series regimes, or have accreditation to ISO 17025, the individually-adapted methods are usually validated to give results equivalent to those given by the gravimetric method specified in ISO 8655-6.

This part of ISO 8655 is applicable to the following types of testing:

- of piston-operated volumetric apparatus for purposes other than type testing or the conformity testing which is required prior to declarations or certification of conformity;
- in user locations, where there may be no suitable balance or facilities to perform the reference method given in ISO 8655-6, but which may have at their disposal a suitable photometer or automatic titrator.

As users have expressed the wish to have alternative tests available, the following observations are given to help them select the most appropriate test methods for their purposes.

- a) **Gravimetric method:** Uncertainty values can increase at volumes significantly below 1  $\mu\text{l}$ , due to increasing balance uncertainty, especially in low humidity areas (where there is increased risk of evaporation) and due to the effects of static electricity. These effects are compensated for through the careful design of the test method specified in ISO 8655-6, which applies to the volume ranges specified in ISO 8655-2 to ISO 8655-5.
- b) **Photometric method:** This may be the method of choice for laboratories having a UV/VIS photometer of suitable wavelength and bandwidth. Uncertainty with this method tends to become lower as test volumes decrease and can be further reduced if the volumes used in dilution steps for the preparation of comparative standards use larger capacity Class A glassware (e.g. 100 ml of chromophore solution diluted to 1 000 ml can lead to lower uncertainty than 10 ml diluted to 100 ml).
- c) **Titrimetric method:** This may be the method of choice of a laboratory already having a titrator with the properties specified in 6.2 and C.4.1. in Annex C. The method is most suited to the testing of piston-operated volumetric apparatus working in the volume range above 500  $\mu\text{l}$ . Again, uncertainty can be reduced if larger capacity Class A volumetric apparatus and larger weights of solid reagents are used to prepare standard solutions.

## ISO 8655-7:2005(E)

If any of these methods is adapted, the expanded uncertainty of measurement needs to be calculated to enable comparison with the reference method. In any case, users will determine that the uncertainty of the chosen method is suitable for their intended purpose.

The tests specified in the ISO 8655 series are intended to be carried out by trained personnel.

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# Piston-operated volumetric apparatus —

## Part 7:

# Non-gravimetric methods for the assessment of equipment performance

**WARNING** — The use of this part of ISO 8655 may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this part of ISO 8655 to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

## 1 Scope

This part of ISO 8655 specifies the photometric and titrimetric determination of errors of measurement of piston-operated volumetric apparatus. The tests are applicable to complete systems comprising the basic apparatus and all parts selected for use with the apparatus, disposable or reusable, involved in the measurement by delivery process.

These non-gravimetric test methods can be applied

- as aids to quality assurance by the supplier,
- as routine quality assurance and routine calibrations by the user, and
- as routine and post-repair testing.

The methods described in this part of ISO 8655 are not applicable as alternatives to the gravimetric reference test methods specified in ISO 8655-6, which gives the only method suitable as a basis for supplier's declarations or independent certification of conformity.

NOTE 1 Metrological requirements for piston-operated volumetric apparatus, especially maximum permissible errors, are specified in ISO 8655-2 to ISO 8655-5.

NOTE 2 For conformity tests or type tests for declaration and certification of conformity, see the gravimetric reference test methods in ISO 8655-6.

## 2 Normative references

The following referenced documents are indispensable for the application 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 648, *Laboratory glassware — One-mark pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

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

ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

ISO 8655-3:2002, *Piston-operated volumetric apparatus — Part 3: Piston burettes*

ISO 8655-4, *Piston-operated volumetric apparatus — Part 4: Dilutors*

ISO 8655-5, *Piston-operated volumetric apparatus — Part 5: Dispensers*

ISO 8655-6:2002, *Piston-operated volumetric apparatus — Part 6: Gravimetric methods for the determination of measurement error*

### **3 Terms and definitions**

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

### **4 Principle**

#### **4.1 Photometric method**

The photometric method of testing piston-operated volumetric apparatus relies upon the relationship between the concentration of a chromophore in solution and its absorbance of light at a specified wavelength, as described by the Beer-Lambert law. The method can use one of two procedures, depending on the needs of the calibration. In both methods, the test volume of liquid to be measured is delivered by the piston-operated volumetric apparatus under test into a known volume of liquid, and the degree of dilution is calculated from photometric measurements.

The first method is suitable for test volumes  $> 20\%$  of the total volume. The total volume depends on the size of the photometric measuring cell and shall be large enough to adequately fill the cell in the UV/VIS photometer. In this method a known volume of solution is prepared having an absorbance near the upper end of the working range of the photometer and its absorbance is measured. The piston-operated volumetric apparatus being tested is used to add an unknown volume of diluent, e.g. water or buffer. The resulting solution is mixed well and its absorbance is measured. The unknown volume delivered can be calculated from the decrease in absorbance.

The second method is suitable for test volumes  $< 20\%$  of the total volume. In this method a known volume of diluent is prepared. The piston-operated volumetric apparatus being tested is used to add an unknown volume of a sample solution of chromophore having known absorbance. The resulting solution is mixed well and its absorbance is measured. The unknown volume delivered is then calculated from the increase in absorbance. Annex A and Annex B give examples for test procedure and calculation.

Other photometric methods can be used, the suitability of which has been validated for the intended purpose.

#### **4.2 Titrimetric method**

The titrimetric test method is suitable for testing volumes of piston-operated volumetric apparatus  $\geq 500\ \mu\text{l}$ . In general, any titration can be used, the suitability of which has been validated for the intended purpose.

For example, a potassium chloride (KCl) solution can be used as test liquid to be dispensed by the device under test into an acidified receiver liquid. The resulting test solution is titrated with silver nitrate ( $\text{AgNO}_3$ ) solution. The equivalence point is determined by potentiometric detection, e.g. with a silver electrode.

If the device under test is a piston burette, known concentrations of potassium chloride in a receiver vessel can be titrated potentiometrically with silver nitrate using the piston burette under test.

Annex C gives an example for the test procedure.

## 5 Reagents

All components of reagent solutions shall be of recognized analytical composition and purity.

### 5.1 Reagents for photometric method

If stock solutions are to be stored for any length of time, they shall be tested for chemical stability, and preservatives added, if needed, to prevent microbiological growth. If the reagents degrade when exposed to light, they shall be stored suitably protected to prevent degradation.

NOTE Instability of reagents when exposed to light can be a major source of uncertainty and a determination of degradation can be necessary.

**5.1.1 Water**, complying with grade 1 in accordance with ISO 3696.

#### 5.1.2 Chromophore solution

The dispensing characteristics of the chromophore solution, which are influenced by material parameters such as surface tension, density and viscosity, shall be as close as possible compared to those of water in order to facilitate correlation between the photometric and the gravimetric test methods. The potential for adsorption of the chromophore on the wall shall be considered. If a discrepancy between the dispensing properties of the chromophore solution and water is noted during the correlation study of the method, that discrepancy shall be included in the uncertainty analysis.

The chromophore chosen shall be completely soluble at the highest concentration required.

NOTE Suitable chromophores are 2,2-azino-di-[3-ethylbenzthiazoline sulfonate(6)] (ABTS, relative molecular mass  $M_r = 547,7$ ), potassium dichromate  $K_2Cr_2O_7$ , Ponceau S and Orange G. An example of a reagent system based on Ponceau S is given in Annex A.

#### 5.1.3 Reagent system

The reagent system, consisting of chromophore, diluent, preservative (if needed) and buffer (if needed) shall be chosen with the following criteria in mind. In all cases the uncertainty of measurement due to the various contributions, e.g. uncertainty of pH, shall be estimated and included in the uncertainty budget.

NOTE An example is given in ISO/TR 16153 <sup>[1]</sup>.

The concentration against absorbance relationship for the reagent system shall be well documented in literature or shall be determined by the user. The wavelength chosen for absorbance measurement shall be at or near an absorbance maximum of the reagent system to minimize the effect of wavelength errors on results.

The reagent system shall either be independent of pH or be buffered to limit pH change to an acceptable range established in the uncertainty budget.

The reagent system shall either be independent of temperature or the results shall be characterized and compensated for temperature.

**5.1.4 Reagent solutions**, to be prepared in concentrations depending on the volumes to be tested.

### 5.2 Reagents for titrimetric method

If the titration of potassium chloride with silver nitrate is used as the titrimetric method, solutions in accordance with 5.2.1 to 5.2.4 shall be used.

**5.2.1 Water**, complying with grade 1 in accordance with ISO 3696.

**5.2.2 Nitric acid**,  $c(HNO_3) = 1 \text{ mol/l}$  or **sulfuric acid**  $c(H_2SO_4) = 0,5 \text{ mol/l}$ .

**5.2.3 Potassium chloride standard solutions**,  $c(\text{KCl}) = 1 \text{ mol/l}$ ,  $c(\text{KCl}) = 0,1 \text{ mol/l}$  and  $c(\text{KCl}) = 0,01 \text{ mol/l}$ .

**5.2.4 Silver nitrate standard solution**,  $c(\text{AgNO}_3) = 0,1 \text{ mol/l}$ .

The solution shall be stored protected from light.

## 6 Apparatus

All apparatus shall be chosen such that the required uncertainty of measurement can be obtained. An example of the calculation of the expanded uncertainty of a photometric method is given in ISO/TR 16153 [1].

All equipment shall be traceable to international or national standards and be of suitable readability, accuracy, reproducibility and stability, consistent with the required expanded uncertainty of measurement.

### 6.1 Photometric method

**6.1.1 UV/VIS photometer**, with adequate resolution, linearity, repeatability, bandpass, absorbance accuracy and wavelength accuracy over the range of absorbances utilized in the method.

An example is given in A.4.1 and Table A.3.

**6.1.2 Measuring cell**, with suitable optical quality.

If its pathlength is not known with sufficient accuracy to meet the requirements of the expanded uncertainty, then a second reagent with known concentrations and absorptivity can be used to enable calculation to compensate for the pathlength's influence on results.

An example is given in A.4.2.

A ratiometric analysis can be applied to determine the unknown volume without reference to measuring cell pathlength.

**6.1.3 Thermometer**

If results are temperature-dependent, the temperature of the solutions shall be measured using a thermometer with uncertainty consistent with the expanded uncertainty of the measurement.

**6.1.4 Volumetric glassware**, Class A

Known volumes of diluent or reagent solutions may be prepared either by using Class A volumetric glassware, or by weighing, in which case the densities of the solutions shall be known.

If piston-operated volumetric apparatus is used for preparation of solutions, it shall conform to the applicable part of ISO 8655 (see Clause 2) and shall be calibrated in accordance with ISO 8655-6.

### 6.2 Titrimetric method

**6.2.1 Complete titration equipment**, comprising burette, e.g. in accordance with ISO 8655-3, and a sensor system for detection of the equivalence point of the chosen titration.

**6.2.2 Electrode**

If the titration of potassium chloride with silver nitrate is used as the titrimetric method, a combined silver electrode should be used for potentiometric indication of the equivalence point. The silver surface of the electrode should, preferably, be coated with AgCl or Ag<sub>2</sub>S (see C.4.2).

**6.2.3 Class A volumetric glassware**, such as one-mark pipettes in accordance with ISO 648 and one-mark volumetric flasks in accordance with ISO 1042.

If piston-operated volumetric apparatus is used for the preparation of solutions, it shall conform to the applicable part of ISO 8655 (see Clause 2) and shall be calibrated in accordance with ISO 8655-6.

#### **6.2.4 Analytical balance**

If the standard solutions according to 5.2.3 and 5.2.4 are prepared by the user, an analytical balance with appropriate performance, such as appropriate minimum mass, shall be used.

### **7 Test conditions**

**7.1** Test room and general conditions should be in accordance with 6.1 and 6.2 of ISO 8655-6:2002.

**7.2** Testing volume and number of measurements per volume to be tested depend upon user requirements. Guidance can be found in 7.1.1 and 7.1.2 of ISO 8655-6:2002.

### **8 Procedure**

#### **8.1 General**

Perform the preparation of solutions and measurements at a stable temperature, preferably 20 °C.

Perform the testing in accordance with the general principles specified in 4.1 and 4.2, and in accordance with the manufacturer's instructions for the equipment specified in 6.1 and 6.2.

#### **8.2 Photometric method**

Two detailed examples for the application of the photometric method, including calculation of the dispensed testing volumes, are given in Annexes A and B. In the first example, a removable measuring cell (also known as cuvette or vial) containing a known volume of diluent is placed into the UV/VIS photometer and the test volume is dispensed into it while it is in the UV/VIS photometer. The absorbance of the mixture is read after mixing.

In the second example (see Annex B), the test volume is dispensed into a container with a known volume of diluent, the solution is mixed, and an aliquot is drawn into a flow cell in a UV/VIS photometer where the absorbance is measured.

In both cases the unknown volume is calculated using the Beer-Lambert law, based on measured absorbances and the diluent volume.

#### **8.3 Titrimetric method**

A detailed example for the application of the titrimetric method, including calculation of the dispensed testing volumes, is given in Annex C. If the titration of potassium chloride with silver nitrate is used as the titrimetric method, solutions shall be chosen as specified in Table 1. Water for the preparation of all solutions shall comply with ISO 3696, grade 1.

**Table 1 — Solutions for the titrimetric test method**

Test volume ml	Test liquid $c(\text{KCl})$ mol/l	Receiver liquid for test liquid		Titration solution $c(\text{AgNO}_3)$ mol/l
		$\text{H}_2\text{O}$ ml	$c(\text{HNO}_3) = 1 \text{ mol/l}$ ml	
0,5 to 1	1	100	2	0,1
> 1 to 10	0,1	100	2	0,1
> 10 to 200	0,01	100	2	0,1

Dispense the test liquid (KCl) with the piston-operated volumetric apparatus under test into the acidified receiver solution, observing the manufacturer's instructions for use and the testing requirements given for individual piston-operated volumetric apparatus in ISO 8655-6:2002, 7.2 to 7.7.

NOTE The special test requirements and the details of the dispensing process for single-channel piston pipettes with air interface are given in ISO 8655-6:2002, 7.2

After careful mixing of the dispensed test liquid with the receiver solution, titrate the resulting solution with 0,1 mol/l silver nitrate solution, observing the manufacturer's instructions for the titration equipment given in 6.2.

## 9 Calculation

From the dispensed volumes of the piston-operated volumetric apparatus under test, the systematic and random errors of measurement and, when appropriate, the uncertainty of measurement shall be calculated using the equations given in ISO 8655-6:2002, 8.4 to 8.6.

## 10 Test report

The test report shall contain, as a minimum, the information specified in ISO 8655-6:2002, Clause 9. The test report shall indicate the applied test method and data regarding the expanded uncertainty of measurement.

## Annex A (informative)

### Example 1 for photometric test method

#### A.1 Objective

This annex describes the details of a photometric test method for measuring microlitre liquid volumes dispensed with piston-operated volumetric apparatus. This example uses replicable vials in a UV/VIS photometer. If this method is chosen, the procedure shall be followed.

#### A.2 Outline of the method

This method is designed to be used with prefilled disposable measuring cells (vials) of unknown pathlength.

A diluent is prepared containing a chromophore with an absorbance peak at wavelength  $\lambda_2$ , but little or no absorbance at a first wavelength  $\lambda_1$ . A stock chromophore solution is prepared by dissolving chromophore in water with resulting absorbance peak at  $\lambda_1$ .

A standard is prepared by accurately diluting several millilitres of this stock solution with diluent. An aliquot of standard solution is dispensed into a vial, and absorbances are measured at both wavelengths.

Disposable glass vials are prefilled with an accurately measured volume of diluent. To initiate a photometric calibration, the cap is removed from one of these vials and it is inserted into the holder of the UV/VIS photometer. Absorbance readings are made at both wavelengths. The reading at  $\lambda_1$  provides a zero for the UV/VIS photometer.

Stock solution is then delivered into the vial using the piston-operated volumetric apparatus under test. The UV/VIS photometer is provided with a mixing means that thoroughly mixes the unknown added volume with the diluent. After mixing, a new absorbance reading is taken at  $\lambda_1$ .

The delivered volume can be calculated based on the Beer-Lambert law using measured absorbances, diluent volume and the dilution ratio used in preparing the standard.

#### A.3 Reagents

All components of reagent solutions shall be of recognized analytical composition and purity.

**A.3.1 Water**, complying with grade 1 in accordance with ISO 3696.

##### A.3.2 Test solutions

All measurements are compared to a calibration measurement of a standard that is prepared using large volumes of the stock solution and the diluent. All dilutions shall be made with a high degree of accuracy, e.g. using Class A volumetric glassware or gravimetric measurement.

All solutions shall be mixed with extreme thoroughness, e.g. 10 inversions to ensure repeatability and accuracy of results.

##### A.3.3 Stock solutions

The dye Ponceau S is dissolved in water. Different concentrations are required, depending on the nominal volumes or selected volumes of the piston-operated volumetric apparatus to be measured. A given

concentration of stock solution can be used over a 5:1 volume measurement range. Concentrations of the stock solutions are chosen so that the absorbances of the diluted samples are within the linear range of the UV/VIS photometer.

EXAMPLE For 5 ml of diluent per sample and 18 mm vials, Table A.1 gives the required stock solution concentration.

Following preparation, stock solutions are filtered through 0,2 µm filters and stored in clean glass containers. The concentrations of the stock solutions are not critical if standards are prepared and read every time that the method is applied. If standards are to be saved, then both they and the stock solution shall be stored so as to avoid evaporation or degradation in tightly capped low-actinic glass bottles, refrigerated, in the dark. The most concentrated stock solutions can precipitate under refrigeration; however, they can be re-dissolved by warming and mixing.

Dilute solutions of Ponceau S and some other dyes display a slight photochromic behaviour when exposed to normal room light. The absorbance may decrease slightly (up to 0,2 %), but then will recover after 10 min in the dark. For the highest accuracy work, all diluted samples should be allowed to equilibrate in the dark before a reading is taken.

Table A.1 — Stock solutions of Ponceau S dye

Stock solution No.	Selected volume µl	Nominal absorbance of stock per 10 mm pathlength	Final solution ml	Ponceau S dye g
1	50 to 200	21,67	1 000	0,476
2	10 to 50	84,4	1 000	1,86
3	2 to 10	418	500	4,58
4	0,5 to 2	1 111	100	2,44

**A.3.4 Diluent**

The diluent is 0,02 mol/l phthalate buffer (pH 6,0) with copper chloride and EDTA added to provide an absorbance peak at 730 nm. The buffer is prepared by dissolving 4,08 g of potassium hydrogen phthalate in approximately 800 ml of water and adding 13,3 ml of 1,0 mol/l NaOH. Then 3,74 g of tetrasodium EDTA and 1,12 g CuCl<sub>2</sub>·2H<sub>2</sub>O are added and dissolved in the solution. The pH is adjusted to pH 6,0 with either 0,1 mol/l HCl or 0,1 mol/l NaOH, as needed. The solution is then filtered through a 0,2 µm filter and made up with water to 1 000 ml. The diluent can be stored for up to one month under refrigeration.

**A.3.5 Standard solutions**

Prepare a standard solution for each concentration of stock solution. The absorbance of a standard should be below the upper end of the linearity range. Standard solutions shall be tightly capped to prevent changes in concentration due to evaporation. Standard solutions may be freshly prepared for each calibration session, avoiding the issue of degradation in storage.

Table A.2 gives the necessary dilutions to make standards. In this table, standard solution No. 1 is created by diluting stock solution No. 1 from Table A.1, etc.

Table A.2 — Standard solutions

Standard solution No.	First dilution		Second dilution <sup>a</sup>		Dilution ratio $R$
	Stock solution ml	Diluent ml	Dilution 1 ml	Diluent ml	
1	5	125	—	—	$3,846 \times 10^{-2}$
2	10	1 000	—	—	$9,901 \times 10^{-3}$
3	2	1 000	—	—	$1,996 \times 10^{-3}$
4	5	1 000	5	100	$2,369 \times 10^{-4}$

<sup>a</sup> The second dilution shall be taken into account when calculating the uncertainty of measurement.

The dilution ratio  $R$  is given by:

$$R = \frac{V_S}{V_S + V_D} \quad (\text{A.1})$$

where

$V_S$  is the volume of stock solution;

$V_D$  is the volume of diluent.

#### A.4 Apparatus

All apparatus shall be chosen such that the required uncertainty of measurement can be obtained. An example of the calculation of the expanded uncertainty of a photometric method is given in ISO/TR 16153 [1].

All equipment shall be traceable to international or national standards and be of suitable readability, accuracy, reproducibility and stability, consistent with the required expanded uncertainty of measurement.

##### A.4.1 UV/VIS photometer, of suitable precision and linearity

The selection of UV/VIS photometer depends on the uncertainty of measurement required by the user. Performance requirements for a typical and for a reference UV/VIS photometer are listed in Table A.3.

Table A.3 — Typical performance requirements of UV/VIS photometers

Parameter	Typical laboratory UV/VIS photometer <sup>a</sup>	Reference grade UV/VIS photometer <sup>a</sup>
Photometric reproducibility at $A = 0$	< 0,001	< 0,000 3
Photometric reproducibility at $A = 0,5$	< 0,001 5	< 0,000 5
Photometric reproducibility at $A = 1$	< 0,0015	< 0,000 5
Photometric reproducibility at $A = 1,5$	< 0,0020	< 0,000 7
Wavelength reproducibility	< 0,5 nm	< 0,2 nm
Photometric stability	< 0,001 h <sup>-1</sup>	< 0,000 2 h <sup>-1</sup>

<sup>a</sup> All specifications are given for 4 nm bandpass and 4 s averaging.

#### A.4.2 Vials and holder

The example given is based on a cylindrical vial with inner diameter of 18 mm as photometer measuring cell. Other vials of adequate optical clarity can be used. The standard shall be read at the same temperature as the unknown. This can be achieved by equilibrating both in the sample compartment of the UV/VIS photometer. The holder should fix the vial in place in the light beam so that it cannot shift during the measurement and mixing process.

### A.5 System linearity

#### A.5.1 General remarks

This method is reliant on the combined linearity of the UV/VIS photometer and the dye system, which therefore shall be measured when the method is validated. Linearity can be measured by making successive dilutions of a sample that starts at the upper end of the absorbance range. The diluent shall be the same as is used in the preparation of unknown and standard solutions.

#### A.5.2 Procedure for measuring linearity

The following procedure should be used to measure linearity.

- a) To 2 volumes of stock solution No. 1 (see Table A.1) add 25 volumes of diluent. This will create a solution with absorbance of approximately 1,6.
- b) Using Class A glassware, accurately measure 2 volumes of this solution, add 1 volume of diluent and mix well.
- c) Repeat, using precisely the same volume in each case, until five successive dilutions are created. These dilutions will span the absorbance range from 0,3 to 1,6.
- d) Let the temperature of the dilutions equilibrate in the dark and read their absorbances in the UV/VIS photometer by successively pipetting the dilutions into a 10 mm cuvette of high optical quality that is fixed in place in the UV/VIS photometer.
- e) Plot the absorbance ratio (measured absorbance divided by the absorbance of the first dilution) against the ratio of dilution. The resulting plot should be linear.
- f) Determine the greatest percentage deviation of any one data point from a linear regression. This deviation, if uncorrected, gives the uncertainty due to nonlinearity.

In the event that the uncertainty due to nonlinearity is not acceptable for a given application, making a greater number of concentrations of stock solutions, with absorbances closer to one another, can restrict the dynamic range of the system and provide greater linearity.

If desired, an algorithm may be constructed to allow compensation for nonlinearity, or a standard may be prepared for each volume of pipette to minimize problems with system linearity.

### A.6 Test procedure

#### A.6.1 General remarks

The following procedure is used to obtain enough data points at each volume to satisfy the laboratory's requirements. In the procedure described below, each data point requires the use of one sample container. Multiple additions of stock solution to a sample container are possible if the dynamic range of the system is

wide enough to provide adequate linearity and the calculation is modified to reflect the changed volume of diluent for each data point.

The standard solution should be read at the same time as the unknown readings, unless adequate data have been collected to demonstrate stability of both reagents and UV/VIS photometer. Ensure that no air bubbles are present in the vial when the readings are taken.

#### **A.6.2 Preparation of sample vials**

Accurately fill sample containers (e.g. 15 ml glass vials) with volume  $V_D$  of diluent. In the example given in A.3.3,  $V_D$  is 5 ml.

A number of these containers can be prepared ahead of time and capped.

All sample containers, e.g. vials, shall be taken from the same production lot to assure vial-to-vial homogeneity of absorbance. The vial-to-vial reproducibility of results shall be tested and included in the uncertainty of measurement, if necessary.

#### **A.6.3 Zero of the UV/VIS photometer**

Place a vial containing 0,02 M pH 6,0 buffer (see A.3.4) into the UV/VIS photometer and zero it at 520 nm and 730 nm.

#### **A.6.4 Absorbances of the standard**

Without disturbing the vial in the UV/VIS photometer, remove the buffer, e.g. using a Pasteur transfer pipette, rinse the vial at least three times with standard solution, and fill the vial with standard.

Read absorbances  $A_{S1}$  and  $A_{S2}$  at 520 nm and 730 nm respectively.

Remove this vial from the UV/VIS photometer and discard.

#### **A.6.5 Absorbances of the diluent**

Remove the cap from a vial of diluent, insert it into the UV/VIS photometer and read absorbances  $A_{D1}$  and  $A_{D2}$  at 520 nm and 730 nm respectively.

#### **A.6.6 Dispensing of test sample**

Using the piston-operated volumetric apparatus being tested, deliver an aliquot of stock solution to the vial of diluent in the UV/VIS photometer.

Observe the applicable dispensing requirements given for each individual piston-operated volumetric apparatus in ISO 8655-6:2002, 7.2 to 7.7.

Activate the mixing mechanism to thoroughly mix the contents.

#### **A.6.7 Absorbance of mixture**

Measure the absorbance  $A_U$  of the resulting mixture at 520 nm.

## A.7 Calculation of the dispensed volumes

The unknown volumes  $V_U$  dispensed by the piston-operated volumetric apparatus under test are calculated using the following formula derived from the Beer-Lambert law:

$$V_U = V_D \left[ \frac{\frac{A_U - A_{D1}}{A_{D2} - A_{D1}}}{\left(\frac{1-R}{R}\right) \frac{A_{S1}}{A_{S2}} - \left(\frac{A_U - A_{D1}}{A_{D2} - A_{D1}}\right)} \right] \quad (\text{A.2})$$

where

$V_U$  is the unknown volume of stock solution dispensed by the piston-operated volumetric apparatus under test;

$V_D$  is the volume of diluent in the sample vials;

$A_U$  is the absorbance of the mixture of stock solution and diluent measured at 520 nm;

$A_{D1}$  is the absorbance of the diluent at 520 nm;

$A_{D2}$  is the absorbance of the diluent at 730 nm;

$R$  is the dilution ratio of stock to diluent that was used in creating the standard;

$A_{S1}$  is the absorbance of the standard solution at 520 nm;

$A_{S2}$  is the absorbance of the standard solution at 730 nm.

A derivation of this equation is provided in ISO/TR 16153 [1].

## Annex B (informative)

### Example 2 for photometric test method

#### B.1 Objective

This annex describes the details of a photometric test method for measuring microlitre liquid volumes dispensed with piston-operated volumetric apparatus. This example uses a flow cell or a conventional cuvette with a pathlength of 10 mm in a UV/VIS photometer. If this method is chosen, the following procedure shall be followed.

#### B.2 Outline of the method

This method is designed around the use of a flow cell in a UV/VIS photometer. All measurements are made ratiometrically to avoid introducing errors due to uncertainties in cell pathlength or the concentrations of sample solutions. This method is useful in environments where a microbalance is impractical, e.g. because of air drafts or vibration.

Stock chromophore solution is prepared by dissolving an appropriate quantity of chromophore in water. One or more diluted standards are prepared from this stock solution using Class A volumetric glassware in accordance with ISO 648 and ISO 1042. In making these dilutions, volumes of stock solution and diluent shall be large enough so that the uncertainty in dilution is small in relation to other sources of uncertainty.

Sample containers are prepared by accurately dispensing diluent into each of them. An unknown amount of stock solution is then added to these containers using the piston-operated volumetric apparatus under test, following which the container is tightly capped and thoroughly mixed. One container is required for each data point.

The absorbance of the standard is read in a flow cell in the UV/VIS photometer, followed by readings of each of the sample containers. According to the Beer-Lambert law, the unknown volumes can be calculated from the volume of diluent, the dilution ratio used in making the standard, and the measured absorbances.

#### B.3 Reagents

All components of reagent solutions shall be of recognized analytical composition and purity.

##### B.3.1 Test solutions

Test solutions shall be prepared in accordance with A.3.2.

##### B.3.2 Stock solutions

Choose the appropriate concentration, in accordance with A.3.3 and Table B.1, so that the absorbances of the diluted samples are preferentially within the range 0,3 to 1,5 in a flow cell of 10 mm pathlength.

Table B.1 — Stock solutions for photometric flow cell method

Stock solution No.	Selected volume µl	Nominal absorbance of stock per 10 mm pathlength	Final solution ml	Ponceau S dye g
1	50 to 200	39	1 000	0,856
2	10 to 50	152	1 000	3,34
3	2 to 10	752	500	8,25
4	0,5 to 2	2 000	100	4,39

### B.3.3 Diluent

The diluent is 0,02 mol/l phthalate buffer (pH 6,0).

Prepare the diluent by dissolving 4,08 g of potassium hydrogen phthalate and 13,3 ml of 1,0 mol/l NaOH in water and making up to 1 l.

Adjust the pH, if necessary, by adding either HCl or NaOH, and filter the diluent through a 0,2 µm filter after preparation. The diluent can be stored for up to one month under refrigeration.

### B.3.1 Standard solutions

Standard solutions shall be prepared in accordance with A.3.5.

## B.4 Apparatus

All apparatus shall be chosen such that the required uncertainty of measurement can be obtained. An example of the calculation of the expanded uncertainty of a photometric method is given in ISO/TR 16153 [1].

All equipment shall be traceable to international or national standards and be of suitable readability, accuracy, reproducibility and stability, consistent with the required expanded uncertainty of measurement.

### B.4.1 UV/VIS photometer

If possible, the UV/VIS photometer should be programmed to make sequential readings at two wavelengths:

- at the 520 nm absorbance peak; and
- at 650 nm, where the dye has no absorptivity.

The program then subtracts the two readings to produce a result that is less influenced by the inadvertent presence of small particles in the solutions.

If the UV/VIS photometer is not programmable, the readings should be taken at two wavelengths and the subtraction done manually.

### B.4.2 Flow cell

The absorbance of all samples shall be read in a flow cell of 10 mm pathlength with a volume not exceeding 1 ml, ensuring that the flow cell is not disturbed during the course of the sample readings.

The sample shall be temperature-controlled within 0,2 °C at the time of the reading.

Using a water bath in conjunction with a jacketed cell holder can control cell temperature. To hasten temperature equilibrium, the sample can be drawn into the cell through a length of small diameter stainless steel tubing, e.g. 30 cm long by 0,1 cm inner diameter, that is jacketed and temperature-controlled by the same water as is circulated through the jacket of the flow cell. The total volume of the flow cell, connecting inlet tubing and inlet filter should not exceed 2 ml.

#### **B.4.3 Sample conditioning**

The sample can be drawn into the flow cell by a peristaltic pump connected to the outlet of the cell. The sample temperature shall be set several degrees below ambient to prevent air from coming out of solution and the consequent formation of small bubbles that cling to the walls of the flow cell, causing erroneous readings.

Preparation of samples and measurement shall be performed at constant temperature, preferably 20 °C. As an additional precaution against bubble formation, reducing pressure to 67 kPa or below while stirring for several hours, can degas water used in preparing solutions.

A stainless steel fabric filter with 60 µm openings may be used to filter the sample as it is drawn into the cell. When aspirating a new 5 ml sample, between 1 % and 5 % carryover of the old sample remains in the cell, mixed with the new sample. The volume of new sample required for adequate flushing shall be determined by experimentation. The presence of carryover can cause an underestimate of the imprecision of results.

#### **B.5 System linearity**

The procedure outlined in A.5 should be used to measure linearity; however, the volume of stock solution should be adjusted in point a) to the following.

- a) To 1 volume of stock solution No. 1 (see Table A.1), add 25 volumes of diluent. This will create a solution with absorbance of approximately 1,5.

For the rest of the procedure, see A.5.2 b) through f).

#### **B.6 Test procedure**

##### **B.6.1 General remarks**

The following procedure is used to obtain enough data points at each volume to satisfy the laboratory's requirements. Each data point requires the use of one sample container.

##### **B.6.2 Preparation of test samples**

Accurately fill sample containers, e.g. 15 ml glass vials, with 5 ml of diluent. A number of these containers can be prepared ahead of time and capped.

Using the piston-operated volumetric apparatus under test, dispense an aliquot of the appropriate stock solution to each sample container.

Observe the applicable dispensing requirements given for each individual piston-operated volumetric apparatus in ISO 8655-6:2002, 7.2 to 7.7.

After thorough mixing, the test samples are allowed to equilibrate in the dark to the same temperature (see B.4.3) as the standard.

##### **B.6.3 Zero of the UV/VIS photometer**

Flush the flow cell thoroughly with diluent until the absorbance no longer changes.

Set the UV/VIS photometer baseline so that readings at both 520 nm and 650 nm are zero.

#### B.6.4 Absorbances of standard and test samples

Draw standard solution into the flow cell and measure the absorbance.

Then draw the test samples into the flow cell one at a time and measure their absorbances.

Following these readings re-measure the standard and re-check the zero to quantify the amount of drift that may have occurred during the reading process.

#### B.7 Calculation of the dispensed volumes

The individual unknown volumes  $V_U$  dispensed with the piston-operated volumetric apparatus under test are calculated using the following formula derived from the Beer-Lambert law:

$$V_U = V_D \left( \frac{A_U R}{A_S - A_U R} \right) \quad (\text{B.1})$$

where

$V_D$  is the volume of diluent used in the sample vial;

$A_U$  is the absorbance of the test sample;

$A_S$  is the absorbance of the standard;

$R$  is the dilution ratio of stock to diluent, that was used in creating the solution standard.

A derivation of this equation is provided in ISO/TR 16153 [1].