INTERNATIONAL STANDARD

ISO 8655-8

First edition
2022-04

Piston-operated volumetric apparatus —

Part 8: Photometric reference measurement procedure for the determination of volume

Appareils volumétriques à piston —

Partie 8: Mode opératoire de mesure photométrique de référence pour la détermination de volumes

Reference number
ISO 8655-8:2022(E)

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Published in Switzerland

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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.

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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 332, *Laboratory equipment*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 8655 series can be found on the ISO website.

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 ISO 8655 series addresses the needs of:

— manufacturers, as a basis for quality control including, where appropriate, the issuance of manufacturer's declarations;

— calibration laboratories, test houses, users of the equipment and other bodies as a basis for independent calibration, testing, verification, and routine tests.

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

Piston-operated volumetric apparatus —

Part 8:
Photometric reference measurement procedure for the determination of volume

1 Scope

This document specifies the photometric reference measurement procedure for the determination of volume of piston-operated volumetric apparatus (POVA). The procedure is applicable to complete systems comprising the basic apparatus with a maximum nominal volume of 5 000 µl and all parts selected for use with the apparatus, disposable or reusable, involved in the measurement by delivery (Ex).

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

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 8655-1:2022, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations*

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

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

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

ISO 8655-9, *Piston-operated volumetric apparatus — Part 9: Manually operated precision laboratory syringes*

ISO/IEC Guide 2, *Standardization and related activities — General vocabulary*

ISO/IEC Guide 99:2007, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in in ISO 8655-1, ISO/IEC Guide 2, and ISO/IEC Guide 99 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 General requirements

When performing calibrations according to the reference measurement procedure described in this document, all provisions and requirements of this document shall be followed or exceeded (e.g., performing 30 instead of 10 replicates per volume). If one or more of those requirements is not followed, conformity to this document shall not be claimed.

5 Test equipment

5.1 General

Measurement equipment for spectrophotometry, weighing, temperature, density, pH, humidity, and barometric pressure shall be traceable to the international system of units (SI) and shall meet the uncertainty requirements of this document.

NOTE An example of the calculation of the expanded uncertainty of the photometric reference procedure is given in ISO/TR 16153[1].

5.2 Spectrophotometer

The visible range spectrophotometer shall meet the performance requirements specified in Table 1 at 520 nm and 730 nm.

Table 1 — Performance requirements of the spectrophotometric system

Parameter	Requirement
Photometric repeatability at A = 0,0 AU [a]	0,000 05 AU
Photometric repeatability at A = 0,5 AU [a]	0,000 05 AU
Photometric repeatability at A = 1,0 AU [a]	0,000 10 AU
Photometric repeatability at A = 1,5 AU [a]	0,000 15 AU
Centre wavelength reproducibility [b]	0,025 nm
Bandwidth reproducibility [b]	0,050 nm
Reproducibility of cuvette attenuance [c]	0,000 10 AU
ND glass calibration standards [d]	
Uncertainty at A = 0,5 AU	0,001 5 AU
Uncertainty at A = 1,0 AU	0,002 5 AU
Uncertainty at A = 1,5 AU	0,003 0 AU

[a] Repeatability to be measured as standard deviation using the same reading procedures, settings and conditions as are used during the photometric volume determination. Adjusting integration time (sample averaging time), bandpass (slit width), and the number of replicate readings are acceptable means of improving the spectrophotometer's repeatability.

[b] Wavelength and bandwidth reproducibility applies to instruments where wavelength and bandwidth are adjustable. It does not apply to fixed-wavelength interference filter instruments.

[c] Cuvette attenuance reproducibility applies to the spectrophotometer and cuvette tested together. An example is given in ISO/TR 16153[1].

[d] Applicable when use of ND glass standard is specified by the manufacturer.

5.3 Cuvette and mixer

The cuvette shall be made of a material with at least 99 % internal optical transmittance at 520 nm and 730 nm. The cuvette shall have an optical path length of 20 mm ± 2 mm. If multiple cuvettes are used, each cuvette shall have a path length within ± 0,2 mm of the chosen nominal.

A mixing mechanism shall be fitted to the cuvette holder of the spectrophotometer, such that the cuvette's contents can be mixed while the cuvette remains seated in the spectrophotometer. Mixing

shall ensure that the liquid contents are mixed to within 0,010 % of complete mixing. Mixing speed shall be sufficient to wash down dye solution deposited on the cuvette side wall.

Mixing mechanisms, such as orbital mixing, a glass-covered magnetic stir bar or a PTFE-covered (polytetrafluoroethylene, PTFE) magnetic stir bar may be used and shall be verified to meet this requirement.

NOTE Complete mixing is achieved when re-mixing and re-measuring the absorbance produces a systematic change no larger than the required value.

5.4 Measuring devices

The minimum requirements for each relevant measuring device are specified in Table 2.

Table 2 — Minimum requirements for measuring devices

Device	Resolution	Expanded uncertainty of measurement (k = 2)
Thermometer for liquids	0,01 °C	0,2 °C
Thermometer for room air	0,1 °C	0,3 °C
Hygrometer	1 % relative humidity	5 % relative humidity
Barometer	0,1 kPa	1 kPa
Timing device	1 s	not applicable

NOTE Acceptable means of measuring the temperature of a solution in a cuvette include a thermistor bead probe immersed in the solution within the cuvette; a suitable contact thermometer on the outside of the cuvette; or a suitable infrared thermometer.

5.5 Equipment used for solution preparation

Solutions shall be prepared by gravimetric or volumetric means.

The liquid components of solutions may be weighed using balances, which shall meet the requirements of Table 3.

For volumetric preparations, class A glassware meeting the maximum permissible errors for narrow neck flasks of ISO 1042 shall be used.

5.6 Balances

Balances used for accurate weighing of dry reagents, preparation of calibrator solutions, and filling of cuvettes shall meet the requirements specified in Table 3.

Table 3 — Minimum requirements for balances

Minimum mass to be weighed g	Resolution (d) mg	Repeatability (s) mg	Expanded uncertainty in use (k = 2) [a] mg
1,0	0,01	0,02	0,04
10	0,1	0,2	0,4
100	1	2	4
1 000	10	20	40

[a] Uncertainty in use is determined according to Reference [2] at the minimum mass listed in the table.

Weighing results for liquids shall be corrected for density, temperature and air buoyancy when determining volume, see Annex A.

5.7 Density meter

Densities of the chromophore solutions shall be measured for each lot of solutions using a temperature-controlled density meter with an uncertainty of 0,000 05 g/ml (k = 2) or better.

5.8 pH meter

The pH meter is used for the preparation of the solutions in 6.3, 6.4, 6.5, and 6.6. It shall be calibrated with reference buffer solutions over a range from pH 4 to pH 7 including pH 6,00 according to manufacturer's instructions. A reference material having a certified value in the range of pH 6,00 ± 0,05 and an uncertainty (k = 2) of 0,02 pH units, or better is required for comparison.

6 Reagents

6.1 General requirements

All components used in the preparation of reagent solutions shall be of at least 99 % analytical purity unless otherwise stated.

NaOH (CAS No. 1310-73-2) and HCl (CAS No. 7647-01-0) solutions may be used for adjustment of pH. The measured pH value of the reagent solutions in 6.3, 6.4, 6.5 and 6.6 shall be compared to the pH 6 certified reference material in 5.8.

6.2 Water

Water (CAS No. 7732-18-5) used for preparing chromophore solutions shall comply with Grade 1 in accordance with ISO 3696:1987.

6.3 Buffer solution

Dissolve 4,08 g of potassium hydrogen phthalate (CAS No. 877-24-7) and 3,81 g of tetrasodium ethylenediaminetetraacetic acid dihydrate (EDTA, CAS No. 10378-23-1) per litre of water, adjust to pH 6,0 ± 0,1, and filter through a 0,2 µm filter.

6.4 Copper(II) chloride solution

Dissolve 1,12 g/l of copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$) (CAS No. 10125-13-0) in the phthalate/EDTA buffer and adjust to pH 6,0 ± 0,1. Filter the resulting solution through a 0,2 µm filter.

6.5 Ponceau S solutions

Dissolve Ponceau S (CAS No. 6226-79-5) in water, adjust to pH 6,0 ± 0,1, and then filter the dye solution through a 0,2 µm filter. Table 4 indicates the amount of Ponceau S dye (which can contain up to 15 % water) per litre of solution. These Ponceau S solutions are used for the preparation of calibrator solutions (see 6.6) and as test liquids.

NOTE 1 Ponceau S solutions prepared according to Table 4 are suitable to measure test volumes from 0,1 µl to 5 000 µl in cuvettes as specified in 5.3.

NOTE 2 When prepared as described in this subclause, the solutions will fulfil the density and viscosity requirements listed in Table 4.

Table 4 — Ponceau S solutions

Ponceau S solution No.	Test volume V_S µl	Ponceau S dye [a] g/1 000 ml	Relative density (vs. H_2O)	Viscosity at 20 °C mPa · s
1	$200 \leq V_S \leq 5\,000$	0,024	1,000 to 1,004	0,9 to 1,1
2	$50 \leq V_S < 200$	0,052	1,000 to 1,004	0,9 to 1,1
3	$10 \leq V_S < 50$	0,165	1,000 to 1,004	0,9 to 1,1
4	$2 \leq V_S < 10$	0,745	1,000 to 1,004	0,9 to 1,1
5	$0,5 \leq V_S < 2$	3,72	1,000 to 1,004	0,9 to 1,1
6	$0,1 \leq V_S < 0,5$	14,9	1,000 to 1,016	0,9 to 1,1

[a] Amounts listed in this table are target values. Actual amounts may vary up to ± 5 % from the target value, provided the same batch of solutions is used for the preparation of calibrator solutions (6.6) and as test liquids (8.3).

6.6 Calibrator solutions

Prepare a calibrator solution for each selected volume V_S to be tested. Mix a measured volume of Ponceau S solution (see Table 4) with a measured volume of copper(II) chloride solution. Determine the volumes of each solution as follows:

Ponceau S solution: use a 10-fold amount of the desired test volume V_S (for n = 10 replicates) and multiply it by the preparation factor given in Table 5.

Copper(II) solution: multiply the volume V_{C0} of copper(II) chloride solution in the cuvette by the preparation factor given in Table 5.

NOTE V_{C0} is defined in 8.3.1.

Table 5 — Examples for calibrator solutions

Test volume V_S µl	Ponceau S solution No.	Preparation factor	Ponceau S solution to measure V_{PS} ml	$CuCl_2$ solution to measure [a] V_C ml
0,1	6	1 000	1	5 000
0,2	6	1 000	2	5 000
0,5	5	1 000	5	5 000
1	5	1 000	10	5 000
2	4	400	8	2 000
5	4	200	10	1 000
10	3	100	10	500
20	3	100	20	500
50	2	100	50	500
100	2	20	20	100
200	1	20	40	100
500	1	20	100	100

[a] Examples in this table are based on V_{C0} = 5 ml.

Calculate the dilution ratio R according to Formula (1):

$$R = \frac{V_{PS}}{V_{PS} + V_C} \quad (1)$$

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.

6.7 Stability of solutions

6.7.1 General

The stability of solutions shall be determined and suitable intervals for recalibration shall be established.

6.7.2 Preservatives

Chromophore solutions may be preserved to prevent microbial growth. Anti-microbial preservatives shall not alter the absorbance properties and density of the solutions beyond the limits given in Table 4. Solutions not preserved shall be recalibrated daily when used.

NOTE Two compounds, which meet this requirement are 5-chloro-2-methyl-4-isothiazolin-3-one (CAS No. 26172–55–4) and 2-methyl-4-isothiazolin-3-one (CAS No. 2682–20–4) at concentrations between 0,000 6 % and 0,001 5 % (mass fraction).

6.7.3 Light sensitivity

The chromophore solutions shall not be used or stored in direct sunlight. Solutions shall be stored in the dark when not in use.

NOTE The specified chromophore solutions are stable during normal use under indoor lighting.

6.7.4 Storage temperature

Preserved solutions may be stored at the test room conditions specified in 7.2.

7 Test conditions

7.1 General

All test equipment shall be operated as specified in the operation manual.

7.2 Test room

Photometric measurements shall be performed in a draught-free room with a relative humidity between 45 % and 80 % and temperature of (20 ± 3) °C with a maximum variation of ± 0,5 °C during the test. Prior to the test, the apparatus to be tested, all test equipment, and test solutions shall have stood in the test room conditions for a sufficient time to reach equilibrium with the test room conditions. The temperature variation of the test room during this equilibration period should not be more than 0,5 °C per hour.

The environmental conditions, air temperature and air humidity, shall be within the specified limits for the test room for at least 2 h before starting the test (minimum equilibration time) and during the test itself.

NOTE It is unlikely that this minimum equilibration time will be less than 2 h and can be considerably longer.

When the POVA is required for use in a country that has adopted a standard reference temperature of 27 °C (the alternative temperature recommended in ISO 384 for such use), this figure shall replace the reference to 20 °C.

7.3 Evaporation

Aliquot containers for test liquids should be of a design that minimizes evaporation. The period of time an aliquot container with test liquid can be uncovered and exposed to ambient conditions of the test room shall be known. Remaining unused aliquots of test liquid shall be discarded at the end of that time period.

Measure the evaporation rate in the local environment and calculate the permissible open period according to Formula (2). Place the aliquot container on a balance and measure the mass lost in one minute. Then divide the total aliquot volume by the evaporation rate, and multiply by an acceptable evaporation limit L_E which shall not exceed 0,05 %.

$$P_O = \frac{W_A L_E}{R_E} \tag{2}$$

where

P_O is the permissible period of time that the aliquot container may be open, in s;

W_A is the mass of test liquid in the aliquot container, in mg;

L_E is the acceptable evaporation limit, in %, not to exceed 0,05 %;

R_E is the measured evaporation rate in the local environment, in $mg \cdot s^{-1}$.

8 Procedure

8.1 General

8.1.1 Summary

In this ratiometric photometry procedure, a cuvette containing a known volume of copper(II) chloride solution is placed into a spectrophotometer and its absorbance is measured. The POVA under test is used to add a volume of Ponceau S test liquid. The resulting solution is mixed well, and the absorbance of the mixture is measured. The volume delivered by the POVA is then calculated as detailed in Clause 9.

8.1.2 Test conditions

At the start and at the end of the measurements, the temperature of the test liquid shall be recorded. The air temperature, the barometric pressure and the relative humidity in the test room shall be recorded.

NOTE The relative humidity is necessary for the stability of the room conditions and is necessary for documentation in the test report.

8.1.3 Test volume

In the case of a fixed-volume POVA, the test volume is the nominal volume. In the case of a variable-volume (user selectable volume) POVA, at least the following three volumes shall be tested:

— nominal volume;

— 50 % of the nominal volume, or the closest possible (if equidistant, use the higher value);

— the lower limit of the useable volume range or 10 % of the nominal volume (whichever is the greater).

8.1.4 Number of measurements per volume to be tested

To determine the volumetric measurement error of POVA according to this document, at least n = 10 replicate measurements for each test volume shall be performed. These measurements are used to calculate the systematic and the random error of measurement in accordance with Clause 9.

8.2 System calibration

8.2.1 General

Calibrate the system at each volume V_S at which the POVA is to be tested with the appropriate calibrator solution according to 6.6.

All cuvettes and solutions for this system calibration shall be isothermal with the temperature at which the absorbance measurements are performed (see 7.2). Record this temperature.

8.2.2 System calibration procedure

Follow the system calibration procedure as follows:

a) Zero the spectrophotometer with phthalate/EDTA buffer;

b) Measure the absorbances $A_{CalC520}$ and $A_{CalC730}$ at 520 nm and 730 nm, respectively, of the copper(II) chloride solution;

c) Measure the absorbance A_{Cal520} at 520 nm of the calibrator solution appropriate for the desired test volume V_S (see 6.6).

The calibration procedure may be performed in a single cuvette, which remains seated in the spectrophotometer throughout steps a) to c). Multiple cuvettes may be used for the above sequence of measurements if the cuvettes meet the attenuance reproducibility requirement specified in Table 1.

8.2.3 Previous calibration

If the system has been previously calibrated, the previous calibration values may be used provided that:

— stability of all solutions has been demonstrated;

— the same batch of all solutions is used;

— the temperature of the measured solution in the cuvette is within ± 0,5 °C of the previous calibration, or a temperature correction is applied.

8.3 Photometric procedure

8.3.1 Preparation of cuvettes

Prepare cuvettes by delivering V_{C0} (between 4,5 ml and 5,5 ml) of copper(II) chloride solution. When preparing several cuvettes, each actual delivered volume shall be within ± 0,03 % of V_{C0} and recorded as actual volume at the test temperature. Formula (A.1) in Annex A shall be used to calculate the actual delivered volume of copper(II) chloride solution.

NOTE Formula (A.1) requires knowledge of the density of the copper(II) chloride solution, which is determined using the density meter specified in 5.7.

These cuvettes may be prepared ahead of time and tightly capped to prevent evaporation.

8.3.2 Zero of the spectrophotometer

Place a cuvette containing phthalate/EDTA buffer into the spectrophotometer and zero its absorbance readings at 520 nm and 730 nm.

8.3.3 Starting absorbances

Uncap one of the cuvettes prepared in 8.3.1, insert it into the spectrophotometer, and measure absorbances A_{C520} and A_{C730} at 520 nm and 730 nm, respectively. Do not remove the cuvette from the spectrophotometer after the measurements. Record the temperature of the chromophore solution at each absorbance measurement.

Care shall be exercised that the cuvette is not moved (e.g. turned in case of round cuvettes) in the cuvette holder, so that there is no change to the optical path. This applies also to 8.3.4.

8.3.4 Dispensing of test liquids

The test liquid shall be delivered into the cuvette following the specific procedures described in 8.4 to 8.10 unless the POVA manufacturer's instructions specify a different volume delivery procedure, in which case this procedure (manufacturer's instructions) may be used. If the manufacturer's instructions are used, this procedure shall be documented in the test report in sufficient detail to allow the test to be replicated.

Upon delivery of the test liquid, completely mix the chromophore solutions in the cuvette without removing it from the spectrophotometer's light path.

8.3.5 Absorbance of the chromophore mixture

Measure the absorbance at 520 nm of the chromophore mixture in the cuvette, A_{M520}.

Record the temperature of the chromophore mixture at each absorbance measurement.

Additional deliveries of test liquid to a cuvette may be made according to 8.3.4. The total of all additions shall not exceed the maximum capacity of the cuvette.

8.3.6 Calculation of the delivered test volume

Calculate the delivered volume of test liquid in accordance with Clause 9.

8.4 Preparation

Leave the POVA under test, test equipment, exchangeable parts, and test liquids to reach thermal equilibrium.

Prepare the cuvettes and spectrophotometer according to the steps in 8.3.1 and 8.3.2. Prepare aliquot containers with the test liquids corresponding to the test volumes to be measured.

If using a variable volume POVA, select the test volume; this setting shall not be altered during the test cycle of all replicate measurements.

If testing a burette or dispenser, place the POVA under test, with its reservoir already filled with test liquid, in such manner so that delivery of the test liquid directly into the cuvette is possible without removing the cuvette from the spectrophotometer. Prime the POVA under test according to manufacturer's instructions in order to remove any air bubbles inside the tubes and valves. Set the delivery velocity according to the manufacturer's instructions. The first drops of liquid might need to be discarded before starting the calibration, if indicated by the manufacturer.

8.5 Single-channel air displacement pipettes (in accordance with ISO 8655-2)

8.5.1 General

In the case of electronic motorised pipettes, aspiration and delivery of test liquid are automatic. The remainder of the procedure is carried out following the steps described in 8.5.2. The user should refer to the operation manual for speed settings of aspiration and delivery.

NOTE More information regarding this type of piston pipettes can be found in ISO 8655-2:2022, Annex B.

Forward pipetting shall always be performed.

8.5.2 Test cycle

Perform the test cycle as follows:

a) Fit the selected tip on the piston pipette;

b) Pre-wet pipette tip five times by aspirating the test liquid appropriate for the test volume and expelling to waste, in order to reach a humidity equilibrium in the air-displacement piston pipette;

c) Depress the plunger;

d) Holding the pipette in a vertical position, immerse pipette tip in the test liquid to the appropriate depth below the surface of the test liquid according to Table 6;

Table 6 — Immersion depths during aspiration, and wait time after aspiration of test liquid [3,4]

Volume µl	Immersion depth mm	Wait time s
≤ 1	1 to 2	1
> 1 to 100	2 to 3	1
> 100 to 1 000	2 to 4	1
> 1 000 to 5 000	3 to 6	3

e) Release the plunger slowly, if hand-operated;

f) Pause for the recommended wait time (see Table 6);

g) Withdraw tip vertically and carefully from the test liquid;

h) Open the lid of the spectrophotometer;

i) Touch the tip on the inside of the cuvette, right above the meniscus, at an angle of approximately 30° to 45°;

j) Depress the plunger and deliver the contents of the pipette into the cuvette;

k) Where applicable, use the blow-out feature of the piston pipette (second stop, based on pipette type) to expel the last drop of liquid before drawing the delivery end of the tip along the inner wall of the cuvette;

l) Draw the tip approximately 8 mm to 10 mm along the inner wall of the cuvette to remove any droplets at or around the tip orifice;

m) Remove the tip from the cuvette;

n) Release the plunger;

o) Close the lid of the spectrophotometer, and mix the solutions in the cuvette;

p) Measure the absorbance $A_{M520}(i)$ at 520 nm, where i = 1 to n (n = 10 or more);

q) Record the temperature of the solution in the cuvette at each absorbance measurement;

r) Repeat steps c) to q) until n = 10 (or more) measurements have been recorded as a series of absorbances $A_{M520}(1)$ to $A_{M520}(n)$;

During the replicate measurements, the pipette tip shall be changed at least once in order to detect the use of damaged or incorrectly manufactured tips and assess the variability of the used tips. For n = 10 replicates, at least two tips shall be used, and the tip shall be changed after 5 measurements. After tip replacement, start at step b).

This tip change is also applicable to positive displacement pipettes with disposable tips (type D2).

The effect of barometric pressure on accuracy should be considered for air displacement pipettes, see ISO/TR 16153 for further details.

The absorbance measurement values obtained shall be evaluated in accordance with Clause 9.

8.6 Multi-channel pipettes (in accordance with ISO 8655-2)

Multi-channel piston pipettes are similar to single-channel pipettes in that they comprise a set of single-volume measuring and delivery units, all operated simultaneously by a single operating mechanism. For the purposes of the volumetric performance test, each channel shall be regarded as a single-channel pipette and tested and reported as such according to 8.5. The obtained values shall be evaluated according to Clause 9.

All channels of a multi-channel pipette should be tested individually to account for the specific design and operational challenges of multi-channel pipettes.

Each channel may be tested individually, one after another, by delivering the test liquid directly into the cuvette of the spectrophotometer. Test liquid is aspirated by all channels together. For the measurement of channel 1, for example, the volume of channel 1 is delivered into the cuvette, while the volumes from all other channels are discarded.

NOTE One way to accomplish this delivery method is by providing a zero-retention flow connection to the cuvette which passes through a dish such that the liquid from the channel under test is delivered into the cuvette while the liquid from all other channels is collected in the dish. Alternatively, a zero- retention transfer vessel can be used to quantitatively transfer the test liquid from the channel under test to the cuvette.

8.7 Positive displacement pipettes (in accordance with ISO 8655-2)

Positive displacement pipettes shall be tested according to 8.5. However, the fivefold pre-wetting of the pipette tip prior to the test only needs to be performed if required by the manufacturer. Only change the pipette tips according to 8.5.2 when testing positive displacement pipettes of type D2 (see ISO 8655-2). Follow the manufacturer's instructions for filling the pipette tip without air bubbles.

The absorbance measurement values obtained shall be evaluated in accordance with Clause 9.

8.8 Burettes (in accordance with ISO 8655-3)

Perform the following test cycle:

a) Load the burette, bubble free, in accordance with the manufacturer's instructions;

b) Measure the background absorbances of the copper(II) chloride solution in the cuvette (8.3.3);

c) Deliver the test liquid from the burette into the cuvette until the selected volume is reached (8.3.4). If the burette is automatically controlled, deliver test liquid until the volume pre-set is reached and no further delivery occurs;

d) Mix the solutions in the cuvette and measure the absorbance A_{M520} at 520 nm (according to 8.3.5);

e) Repeat the test cycle until n = 10 (or more) measurements have been recorded.

When testing partial volumes of the nominal volume of the burette, the piston shall not be reset to the initial position (zero) prior to the next measurement. Ensure that the upper volume limit of the piston, and thus the nominal volume of the burette, are not exceeded when dispensing a partial volume.

The absorbance measurement values obtained shall be evaluated in accordance with Clause 9.

8.9 Dispensers (in accordance with ISO 8655-5)

Perform the following test cycle:

a) Load the dispenser, bubble free, in accordance with the manufacturer's instructions;

b) Measure the background absorbances of the copper(II) chloride solution in the cuvette (8.3.3);

c) Deliver the test liquid from the dispenser into the cuvette (according to 8.3.4);

d) Mix the solutions in the cuvette and measure the absorbance A_{M520} at 520 nm according to 8.3.5;

e) Repeat the test cycle until n = 10 (or more) replicate measurements have been recorded.

Due to the large effect of piston speed on the measuring result, any information contained in the operation manual regarding piston speed is particularly important (e.g. selection of the speed appropriate for water with electronic motorised apparatus).

During operation, ensure that the piston does not hit the stroke limits.

For multiple delivery dispensers (see ISO 8655-5), do not reset the piston between each of the n test cycles to its initial position if there is sufficient test liquid remaining to deliver the next aliquot.

The absorbance measurement values obtained shall be evaluated in accordance with Clause 9.

8.10 Syringes (in accordance with ISO 8655-9)

8.10.1 General

Syringes are instruments used for delivering liquids or gases and can be used for total or partial delivery. The procedure in this document applies to the delivery of liquids only.

8.10.2 Test cycle

Perform the following test cycle after priming the syringe to remove all air bubbles:

a) Aspirate test liquid into the syringe until the fiducial line slightly exceeds the graduation line of the volume to be tested;

b) Ensure that no air bubbles form when aspirating test liquid in step a). Adjust the plunger until the fiducial line corresponds to the graduation line of the selected volume to be tested; remove any droplet at the end of syringe tip;

c) Measure the background absorbances of the copper(II) chloride solution in the cuvette (8.3.3);

d) Deliver the contents of the syringe into the cuvette, touching the delivery end of the syringe tip against the inside wall of the cuvette just above the liquid surface at an angle of approximately 30° to 45° (8.3.4);

e) Draw the syringe tip approximately 8 mm to 10 mm along the inner wall of the cuvette to remove any droplets at or around the tip orifice;

f) Mix the solutions in the cuvette and measure the absorbance at 520 nm in accordance with 8.3.5;

g) Repeat the test cycle until n = 10 (or more) replicate measurements have been recorded.

The absorbance measurement values obtained shall be evaluated in accordance with Clause 9.

9 Evaluation

9.1 Calculation of volume

9.1.1 Calibration constant

The calibration constant K_j for each batch of solutions is calculated using Formula (3). Absorbance values are obtained from the measurements in 8.2. The dilution ratio R is calculated according to Formula (1).

$$K_j = \frac{1}{R_j}\left(\frac{A_{\mathrm{Cal520},j} - A_{\mathrm{CalC520}}}{A_{\mathrm{CalC730}} - A_{\mathrm{CalC520}}}\right) \tag{3}$$

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 according to Formula (1);

$A_{\mathrm{Cal520},j}$ is the absorbance of the Ponceau S calibrator solution 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.

This calibration constant is used in subsequent calculations.

9.1.2 Volume of test liquid

After each delivery i of test liquid, the cuvette contains a total volume of delivered test liquid $V_T(i)$. Calculate $V_T(i)$ according to Formula (4). Absorbance values are taken from 8.3.3 and 8.3.5.

$$V_T(i) = V_{C0}\frac{\dfrac{A_{M520}(i) - A_{C520}}{A_{C730} - A_{C520}}}{K_j - \dfrac{A_{M520}(i) - A_{C520}}{A_{C730} - A_{C520}}} \tag{4}$$

where

$V_T(i)$ is the total volume of test liquid 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 (3);

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

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

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

9.1.3 Temperature correction

If the cubic expansion coefficient γ for the POVA is known, as specified by the manufacturer or measured with sufficient reliability by other means, the volume may be corrected to the reference temperature using Formula (5).

$$V_{T,ref}(i) = V_T(i) \times [1 - \gamma(t_L - t_{ref})] \quad (5)$$

where

$V_{T,ref}(i)$ is the temperature-corrected volume after the i-th delivery;

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

t_L is the test temperature;

t_{ref} is the reference temperature.

If the cubic expansion coefficient γ is not known with sufficient reliability, volumes calculated by Formula (4) may be used for calculations in 9.1.4, 9.2, and 9.3.

9.1.4 Mean volume

Calculate the mean volume $\bar{V}$ as shown in Formula (6).

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

where

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

$V_T(n)$ is the total volume of test liquid after the n-th delivery.

9.2 Systematic error of measurement

Calculate the absolute systematic error of measurement e_S of the POVA using Formula (7):

$$e_S = \bar{V} - V_S \quad (7)$$

where

e_S is the systematic error of measurement, expressed in units of volume;

V_S is the selected test volume at the POVA under test.

This systematic error of measurement may be expressed in percent, using Formula (8):

$$\eta_S = \frac{(\bar{V} - V_S)}{V_S} \times 100\,\% \quad (8)$$

where η_S is the relative systematic error of measurement, expressed in percent;

In the case of fixed volume POVA, the selected test volume V_S is the nominal volume.