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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

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Foreword

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

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

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

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