Pilot study on calibration of micropipettes using the photometric method

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Abstract. Micropipettes, or piston-pipettes, are used in analytical laboratories for medical applications when it is necessary to deliver liquid in the range of the microliter. In order to determine the exact delivered volume of each micropipette it is necessary to calibrate them using the appropriate methods described in ISO 8655[1]. One of the methods is the photometric method which uses a high-resolution photometer and colorimetric solutions to determine the volume delivered by a micropipette. Considering that there is still some lack of knowledge about the performance of this method by the National Metrology Laboratories, it was organized, by IPQ and Artel, a pilot study to verify the applicability of this method in each laboratory.

1. Introduction

During the EURAMET TC F volume subgroup meeting, in 2017, it was discuss the possibility of organizing an interlaboratorial comparison on the calibration of micropipettes using the photometric method [1]. Artel, one of the manufactures of a photometer, volunteered to cooperate with EURAMET in this comparison – EURAMET project 1425. Since the majority of the laboratories do not have this method implemented it was decided to register this project as a pilot study. Seven NMI’s decided to participate, IPQ – Portugal, FORCE – Denmark, RISE – Sweden, CMI – Czech Republic, MIRS – Slovenia, GUM – Poland and EIM – Greece. Artel supplied the photometer, the micropipettes and the tips. Training was provided to the participants prior to the measurements.

The Volume and Flow Laboratory of Portuguese Institute for Quality (IPQ) , acting as the pilot laboratory, performed the initial and final measurements of the micropipettes.

2. The instrument

The selected instruments are three fixed volume Eppendorf pipettes (see figure 1) and one variable volume Rainnin pipette (see figure 2). The micropipettes use a removable plastic tip to aspirate and deliver the liquid. The Photometer (PCS – figure 3), the computer and software were supplied by Artel and training was provided prior to the measurements.

The micropipettes used for this comparison are essentially of plastic material with a coefficient of thermal expansion of $2.4 \times 10^{-4}/^\circ C$ [2].
3. Photometric method

The photometric method uses a high-resolution photometer and colorimetric solutions to determine the volume delivered by a micropipette [3]. It is a ratiometric method; the volume of the liquid delivered is determined by comparison with a larger reference volume of diluent solution. The method is described in ISO 8655-7[1].

The basic principle behind photometric measurement is the conservation of mass. Two additional assumptions are also made to allow the photometric method to be used easily for volume measurements: conservation of volume and the Lambert-Beer Law [3].

In the dual-dye ratiometric photometry two colorimetric solutions are used. Each solution (one red, one blue) has an absorbance peak at a specific analytical wavelength. The basis of this technique is the following: an unknown volume of red dye is delivered into a vial containing a known volume and concentration of blue dye. The concentration of the red dye is also known and the ratio between the two concentrations is a calibration factor for the method. After mixing, the change in absorbance of the resulting volume can be calculated as a ratio. The equation that describes this measurement principle is the following:

\[
\frac{A_S}{A_B} = \frac{V_s}{V_b} K
\]  

(1)

Where,

\( A_S/A_B \) is the absorbance ratio measured in the Photometer

\( K \) is the calibration factor for the dyes

\( V_b \) is the volume of the blank solution

\( V_s \) is the volume delivery to be determined

4. Results

Four micropipettes were tested, with the following volume capacities, 100 \( \mu \)L, 10 \( \mu \)L, 1 \( \mu \)L and 0.1 \( \mu \)L and the procedure was described in detail in the project protocol [4].
4.1. Determination of the reference value
To determine the reference value the formula of the weighted mean is used, by means of the inverses of the squares of the associated standard uncertainty are the weighting factors [5]:

\[
y = \frac{x_1/u^2(x_1) + \ldots + x_n/u^2(x_n)}{1/u^2(x_1) + \ldots + 1/u^2(x_n)}
\]  
(2)

To determine the standard uncertainty \(u(y)\) associated with \(y\) it is used the following expression:

\[
u(y) = \sqrt{1/u^2(x_1) + \ldots + 1/u^2(x_n)}
\]  
(3)

4.2. Volume results
The results of all laboratories and corresponding uncertainties for the four micropipettes are presented in the following figures. For the determination of the reference value only one result from IPQ was considered.

**Figure 4**- Volume results for the 100 \(\mu\)L micropipette  
**Figure 5**- Volume results for the 10 \(\mu\)L micropipette  
**Figure 6**- Volume results for the 1 \(\mu\)L micropipette  
**Figure 7**- Volume results for the 0.1 \(\mu\)L micropipette

From the analysis of the IPQ results it can be verify that the micropipettes were stable during the comparison.

Regarding the participant results there is 88% results that are consistent with the reference value. There was one inconsistent result for each volume and it was not always the same NMI.
5. Uncertainty determination

The uncertainty of pipette calibration for all participants was estimated according to the Guide to the Expression of Uncertainty in Measurement (GUM) [6].

The main contributions for the standard uncertainty of the photometric method are: the repeatability of the measurements, the photometer calibration, the photometer resolution, the solutions and the reproducibility.

Table 1. Uncertainty components in the calibration of a micropipette using the photometric method

| Source / Symbol | Standard uncertainty component | Evaluation process | Evaluation type | Distribution |
|-----------------|-------------------------------|--------------------|-----------------|--------------|
| PCS             | u(PCS)cal                     | Calibration        | A               | Normal       |
|                 | u(PCS)res                     | Resolution         | B               | Rectangular  |
| Solutions       | u(Sol)cal                     | Calibration        | A               | Normal       |
| Repeatability   | urepe                         | Mean standard deviation | A           | Normal       |
| Reproducibility | urepr                         | Mean standard deviation | A           | Normal       |

Some variation can be found in the expanded uncertainty declared by some participants which is mainly due to the repeatability of the measurements.

6. Conclusions

IPQ has implemented the photometric method in its laboratory in 2016 [7], following a bilateral comparison in the frame of EURAMET project 1353 which supported the CMCs publication at the BIPM webpage. This method allowed IPQ increase the range and to reduce the uncertainty claims in the calibration of micropipettes with a volume lower than 100 µL. In order to verify that this can be achievable by other laboratories, it was performed a pilot study by seven Europeans NMI’s. Four micropipettes were calibrated at different nominal volumes.

The obtained results were 88 % consistent with the reference value for all the micropipettes. There was some variation found in the expanded uncertainty declared by the participants and that was mainly due to the repeatability of the measurements. For the majority of the participants this was a fist contact with a new method and it is expect that some variation in results and uncertainty would arise.

References

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