On the microvolume measurement from 0.1 μL up to 100 mL using a microsyringe

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Abstract. The theme of this work involves the measurement of small volumes of liquids, comparing results using the gravimetric method in scales with different resolution. The main objective of this study is to characterize the behaviour of microsyringes during the measurement of microvolumes in the range between 0.1 and 1 μL by the gravimetric method, with metrological traceability, and compare with the results in the range up to 100 μL. The methodology is based on the method described in ISO 8655-6, where the total value of the measurement is considered. It solves a problem for measurements below 1 μL due to the metrological traceability of volume measurements by the gravimetric method is fundamentally linked to the mass, whereby a conversion from volume to mass 1 μL is approximately 1 mg. The smallest standard weight has a nominal value of 1 mg, so if only the dispensed liquid value were considered, there would be no metrological traceability for measurements with volumes below 1 μL. Eleven measurements are made at each point, in order to obtain the average of the measurements. However, each of the eleven measurements is independent, that is, in the first measurement the value of the empty weighing container is recorded and then the mass value of this container with the liquid that was dispensed is recorded. Results were presented and concluded the analysis. For consolidating the results, simulations of values were performed to verify a possible improvement if a scale with a resolution of 0.0001 mg and its respective measurement uncertainty were used.

1. Introduction

The measurement of liquid volume is regular activity in test laboratories, chemical laboratories and biological laboratories, which most often measure small volumes and require high accuracy. In industrial processes, oil refineries or in people's daily lives, measuring the volume of liquids also happens very often. Regardless of the use of the volume measurement instrument or its application, metrological reliability is preponderant. Even on micrometric scales, small deviations are impactful and trigger dubious results, often being the main cause of false positives or false negatives.

With the advancement of research in nanotechnology and the need for results with metrological reliability, there is a great demand in the market for microvolume calibration in the range of 0.1 μL to 1 μL where “micropipettes or piston pipettes are used to perform the most volume measurements in areas such as health, chemistry, biology, pharmacy and genetics” (Batista et al., 2007, 2018). ISO 8655-2 standard specifies metrological requirements, maximum permissible error values, and

requirements for marking and information to be provided to users, for the respective micropipettes used. For calibration of these micropipettes by the gravimetric method, ISO 8655-6 is used as a reference.

The theme of this work involves the measurement of small volumes, more precisely volumes in the range of 0.1 µL to 100 µL, comparing their results using the gravimetric method in scales with different resolution.

The main objective of this study is to characterize the behavior of microsyringes during the measurement of microvolumes in the range between 0.1 and 1 µL by the gravimetric method, with metrological traceability, and compare with the results in the range up to 100 µL.

2. State of Art

Another instrument widely used for measuring microvolume is the microsyringe. Syringes are basically made up of a plunger that runs inside a tube and that aspirates and dispenses the fluid (which can be liquid or gaseous). Its use occurs in several segments, such as chromatography, for the application of drugs and measurement of the volume of liquids.

Its use dates back many centuries, but for microvolume measurement it was an advance that occurred in the late 1940s. According to Ettre, 2002, the microsyringe developed by Clark Hamilton was lead shielded and was used for manipulation of radioactive isotopes. From the 1950s Hamilton's efforts turned to microvolume measurement with a focus on chromatography.

The microsyringe is a highly accurate instrument, as it is made up of very fine channels that allow you to manipulate the amount of liquid with great precision. Its use requires great care, as most models are made of glass, which increases the probability of damage, and its handling allows thermal exchange between the user's hand and the liquid. It is common to see parallax related measurement errors as graduation markings are often very fine which requires good skill and user experience. Figure 1 shows a microsyringe with a maximum volume of 5 µL and resolution of 0.05 µL. It is possible to observe how thin and close the markings of the graduations are.

Figure 1: Microsyringe

Gravimetric Method

The gravimetric method is the most used procedure adopted in laboratories and national metrology institutes for volume calibration. It covers all measurement ranges, from microvolumes to large capacity vessels, extending to users of these calibrated instruments, due to the familiarity of the method, since it is widely disseminated and, in a way, easy to apply. Another factor that favors its use is that the instruments used for these measurements are generally shared in the laboratory's daily routine that is, they do not need to be dedicated to measuring volume, which becomes advantageous when we think about the costs related to the measurement.

Depending on the application, some type of errors can be negligible. For example, industrial applications and clinical analysis. However, in a laboratory of glassware calibration, where a high degree of accuracy is required, this error is extremely representative (Barbosa et al, 2011).

According to Sampaio et al, 2019 the choice of the points at which the scale will be calibrated must be made with a focus on the use of the equipment and the information contained in the calibration certificate must be carefully analyzed and properly implemented, with the aim of correctly using its values.

3. Methodology

The methodology used is a measurement routine using a microsyringe. The difference in mass between the full container and the empty container and the subsequent conversion of the mass value into volume. The difference is that with the microsyringe, the meniscus must be adjusted at the point to be measured, before dispensing the volume.

Figure 2a: Sartorius Scale

Figure 2b: Mettler Toledo Scale

The method developed for this work was based on the method described in ISO 8655-6, where the total value of the measurement (container added to the liquid) will be considered. In addition to characterizing the correct way of using the measured values, it solves a problem for measurements below 1 µL due to the metrological traceability of volume measurements by the gravimetric method is fundamentally linked to the mass, whereby a conversion from volume to mass 1 µL is approximately 1 mg. As already discussed in this work, the smallest standard weight has a nominal value of 1 mg, so if only the dispensed liquid value were considered, there would be no metrological traceability for measurements with volumes below 1 µL.

It is important to point out that the tare function is generally a good option for general uses of the scale, which minimizes time and helps in several applications, but for volume measurement it requires due attention so that it is possible to obtain valid values like this to provide metrological traceability. In this work all measurements were made without using the tare function.

The instruments used to determine the volume in both methods are:

- Hamilton Microsyringe, nominal value: 5 µL, one-division value: 0.05 µL;
- Hamilton Microsyringe, nominal value: 100 µL, one-division value: 1 µL.

A methodology was also used to minimize the effect of liquid evaporation during calibration. According to ISO 8655-6, for volume measurements with a nominal value below 50 µL, ways to minimize the mass lost through evaporation must be considered and this loss must be considered as a source of uncertainty in the volume calculation.

Eleven measurements are made at each point, in order to obtain the average of the measurements. However, each of the eleven measurements is independent, that is, in the first measurement the value of the empty weighing container is recorded and then the mass value of this container with the liquid that was dispensed is recorded. Even so, the contribution in the calculation of uncertainty regarding evaporation continues to exist but becomes less significant than when calculated as described in Euramet CG-19, 2015.

To guarantee the reliability of the results, the temperature of the purified water used in the calibrations must be 20 °C ± 1 °C, which cannot differ ± 0.3 °C from the temperature at which the

specific mass of the water and the variation were determined. Maximum temperature of this water at the time of calibration must not exceed ± 0.3 °C. Environmental conditions in the laboratory must be monitored during calibration. The ambient temperature during should be 20 °C ± 1 °C. Relative air humidity should remain in the range of 55% to 70%. Before any measurement, microsyringe remains in the laboratory for thermal stabilization for at least 1 h, minimizing problems with variation in the dispensed volume.

3.1 Volume determination

It consists of converting the measured mass value to the volume of liquid dispensed by the microsyringe, as described in equation (1).

$$V = \frac{M}{(\rho_w - \rho_a)} * \left(1 - \frac{\rho_a}{\rho_p}\right) * (1 - \alpha(T - 20)) \quad (1)$$

Where, V is the volume in mL; M is the mass of liquid in the container in g, where M=M_full-M_empty; ρ_w is the specific mass of the liquid used in the calibration, in g/mL; ρ_a is the specific mass of air during calibration, in g/mL; ρ_p is the specific mass of the weight used in the scale calibration, in g/mL; α is the volumetric expansion coefficient of the container material in $〚°C〛^{(-1)}$; T is the temperature of the liquid in °C. The standard uncertainties that are multiplied by the sensitivity coefficients for determining the volume uncertainty are presents in Eq. (2) to (10).

Scale calibration uncertainty ($u(M_1)$)

$$u(M_1) = \sqrt{\left(\frac{U(M)}{k}\right)^2} \quad (2)$$

Where, U(M) is the expanded uncertainty of the scale calibration at the measurement point; k is the coverage factor expressed in the scale calibration certificate.

Uncertainty of scale resolution ($u(M_2)$)

$$u(M_2) = \sqrt{\left(\frac{r}{2*\sqrt{3}}\right)^2} \quad (3)$$

Where, *r* is the resolution of the scale

Evaporation rate uncertainty ($u(M_3)$)

$$u(M_3) = \sqrt{\left(\frac{m_P}{\sqrt{3}}\right)^2} \quad (4)$$

Where, m_P is the lost mass for the calculation of evaporation.

Uncertainty of the specific mass of water ($u(\rho_w)$)

$$u(\rho_w) = \frac{U(\rho_w)}{k} \quad (5)$$

Where, $U(\rho_w)$ is the expanded uncertainty of the density of water; k is the coverage factor obtained in the calculation of the uncertainty of the specific mass of water.

Uncertainty of specific mass of air ($u(\rho_a)$):

$$u(\rho_a) = \frac{U(\rho_a)}{k} \quad (6)$$

Where, $U(\rho_a)$ is the expanded uncertainty of the density of air; k is the coverage factor obtained in the calculation of the uncertainty of the specific mass of air.

Uncertainty of the specific mass of the reference standard weight ($u(\rho_p)$):

$$u(\rho_p) = \frac{U(\rho_p)}{\sqrt{3}} \quad (7)$$

Where, $U(\rho_p)$ is the uncertainty of the specific mass of the standard weight used in the calibration of the scale. The value of this uncertainty is available in OIML R111-1.

Uncertainty of the volumetric expansion coefficient ($u(\alpha)$):

$$u(\alpha) = \frac{U(\alpha)}{\sqrt{3}} \quad (8)$$

Where, $U(\alpha)$ is the uncertainty of the volumetric expansion coefficient of the container.

Temperature gauge uncertainty ($u(T)$):

$$u(T) = \sqrt{\left(\frac{U_{(Termômetro)}}{k}\right)^2 + \left(\frac{r}{2*\sqrt{3}}\right)^2} \quad (9)$$

Where, $U_{Termômetro}$ is the expanded uncertainty of the calibration of the thermometer; k is the coverage factor expressed in the calibration certificate of the thermometer; r is the resolution of the thermometer.

Uncertainty of repeatability ($u(S_V)$):

$$u(S_V) = \frac{S_V}{\sqrt{n}} \quad (10)$$

Where, S_V is the standard deviation to volume measurements; n is the number of measurements.

Operator Uncertainty ($u(op)$):

For this source of uncertainty there will be a change according to the instrument to be calibrated. For microsyringe calibration, equation (11) will be used.

$$u(op) = \frac{\Delta i}{\sqrt{24}} \quad (11)$$

Where, Δi is the difference between the highest and lowest value obtained among the measurements performed with the equipment at the point being calibrated.

4. Results and Discution

Measurements were made using the gravimetric method at the Inmetro Fluids Laboratory using a scale with a resolution of 0.01 mg, (Sartorius), Figure 2a, and at the company Mettler Toledo using a scale with a resolution of 0.001 mg, Figure 2b. In both laboratories, the environmental conditions for carrying out the calibrations were in accordance with the specifications previously described in Gravimetric method. Tables 1 and 2 present results of measurements in a 0.01 mg resolution scale for the range 5 µL (Table 1) and 100 µL (Table 2).

For each calibrated instrument, a table is presented where the measurement results and the respective uncertainties in unit volume are expressed. (µL) and in percentage value (%). This makes it easier to assess the real impact of uncertainty on the calibration result.

Table 1 presents the results for microsyringe calibration in the rage of 5 µL. Even though the microsyringe is a measuring instrument that allows lower uncertainties when compared to the results using a micropipette, the uncertainty values are significantly high at points below 1 µL, this is justified by the influence of the scale.

Table 2 presents the results of the microsyringe calibration of 100 µL. Even with smaller uncertainties when compared to the measurement using a micropipette, the source of uncertainty that most impacts these measurements is related to the handling of the microsyringe by the operator (u_{op}). Two others that are at secondary levels of influence are related to the scale used and the repeatability of the measurements.

Table 1: Microsyringe Results for 5 µL with scale resolution 0.01 mg.

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	v*eff*
0.1	0.10	0.04	41.2%	2.00	1323
0.2	0.20	0.04	21.3%	2.00	1323
0.5	0.50	0.04	8.8%	2.00	1441
1	0.99	0.04	4.5%	2.00	1420
2	1.98	0.04	2.2%	2.00	1426
5	5.00	0.05	0.9%	2.00	1422

Table 2: Microsyringe Results for 100 µL with scale resolution of 0.01 mg.

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	v*eff*
10	10.29	0.10	0.97 %	2.01	484
20	20.35	0.11	0.55 %	2.00	560
50	50.88	0.08	017%	2.01	451
100	101.51	0.08	0.07 %	2.00	1081

In the case of the microsyringe of 100 µL the resolution of the scale used has less influence than the uncertainty of the scale calibration certificate, when compared with the results of the micropipette of 100 µL. Tables 3 and 4 present results of measurements in a 0.001 mg resolution scale for the range 5 µL (Table 3) and 100 µL (Table 4).

Table 3: Results microsyringe 5 µL with scale resolution of 0.001 mg.

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	v*eff*
0.1	0.106	0.012	11.7%	2.01	374
0.2	0.204	0.012	6.0%	2.01	449
0.5	0.507	0.015	3.0%	2.01	456
1	1.009	0.018	1.8%	2.01	287
2	2.005	0.019	1.0%	2.01	252
5	5.013	0.040	0.8%	2.02	143

Table 3 presents the results of the calibration of the 5 µL microsyringe and better results are observed, with uncertainties with values reasonably within the expected range. As the greatest influence is the handling of the microsyringe by the operator (u_{op}) followed by the repeatability of the measurements, it is understood that it is possible to reduce the uncertainty in this range by improving the accuracy of the measurements. In this case, improving the scale calibration uncertainty with a resolution of 0.001 mg (using standard weights with accuracy class E_1) or improving the resolution to 0.0001 mg, would result in a non-significant uncertainty reduction when we analyze the cost involved in these changes.

Table 4: Results microsyringe 100 µL with scale resolution of 0.001 mg.

Nominal Value (µL)	Average Value	U (µL)	U (%)	*k*	v*eff*
10	10.291	0.026	0.25 %	2.01	231
20	20.327	0.049	0.24 %	2.00	238
50	50.793	0.070	0.14%	2.01	413
100	101.531	0.073	0.07 %	2.01	403

Table 4 presents the results of the calibration of the 100 µL microsyringe. Analyzing the complete result, it was possible to observe that from 50 µL the resolution of the scale does not influence so significantly in the expanded uncertainty, see that from this point on the results are very close when compared with the results using the scale with resolution of 0.01 mg. Table 5 presents the comparison of results from 0.1 µL to 100 µL with 0.01 mg and 0.001 mg scales.

Table 5: Comparison of results of microsyringe on scales with different resolutions.

Nominal Value (µL)	U scale resolution 0.01 mg	U scale resolution 0.001 mg
0.1	39.27 %	11.73 %
0.2	20.64 %	6.03 %
0.5	8.37 %	3.01 %
1	4.47 %	1.82 %
2	2.18 %	0.97 %
5	0.92 %	0.80 %
10	0.97 %	0.25 %
20	0.55 %	0.24 %
50	0.17 %	0.14 %
100	0.07 %	0.07 %

In Figure 3, the importance of the scale with a resolution of 0.001 mg in the microsyringe calibration below 1 µL is evident. It is easy to observe that the difference in the results between the two scales is quite large at the 0.1 µL point and decreases, as already observed and discussed in the micropipette calibration, with the increase in the nominal value.

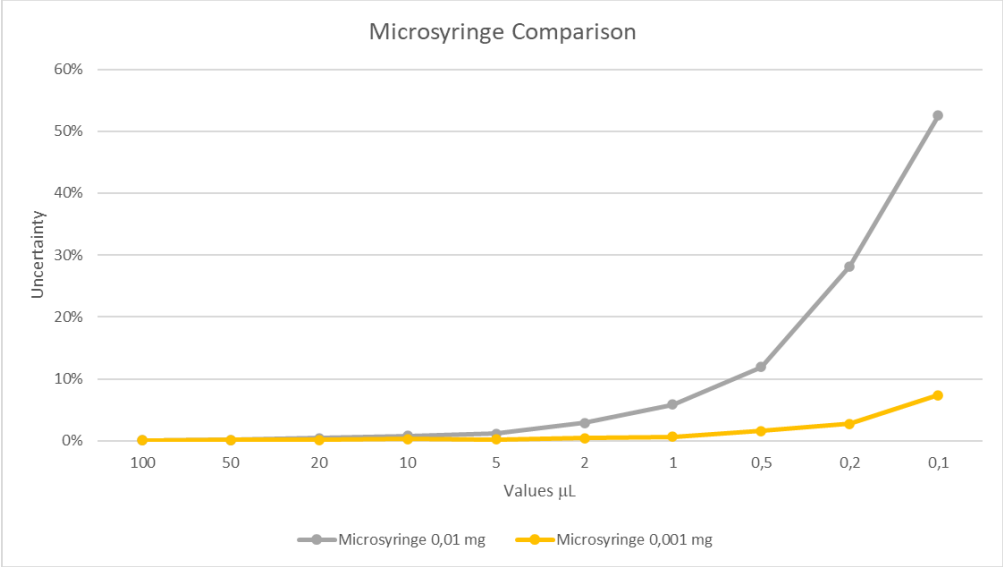

Figure 3: Comparison between microsyringes volume measurems – 0.01 mg and 0.001 mg scales.

After concluding the analysis and consolidating the results, simulations of values were performed to verify a possible improvement if a scale with a resolution of 0.0001 mg and its respective measurement uncertainty were used. The result was that at all measurement points the simulated value did not improve by more than 1% of the expanded uncertainty. It is therefore concluded that the investment in a scale with greater accuracy than 0.001 mg for microvolume calibration up to 0.1 µL is

not necessary. If the laboratory wants to improve its results, it will have to improve other sources of uncertainty that, in most cases, are related to the operation of the instrument (micropipette or microsyringe) and the improvement in the repetition of measurements.

5. Conclusions

Analyzing the results in Table 5, the scale resolution has a direct influence up to the 5 µL point and an indirect influence provided better repeatability up to the 50 µL point. It is worth mentioning that just having a scale with a resolution of 0.001 mg available for microvolume calibration does not solve all problems, it is necessary that it be calibrated using standard weights with accuracy class E1, according to OIML R111-1, thus providing a low expanded uncertainty (which is used as a source of uncertainty in volume calculation). In addition, the measurement with a microsyringe makes it possible to reach lower uncertainties, but the person performing the calibration needs to have training and experience in using this instrument, otherwise it is possible that the results match those of the micropipette. Results of the Table 5, comparing results of microsyringe on scales with different resolutions are presented in plotted form, Figure 3.

In all tables of measurement results it is possible to observe that the relative values of the expanded uncertainty (U%) decrease coherently as the nominal value is increased.

The scale that has the specific model for microvolume calibration, as the container where the measured volume is dispensed has the same mass value when compared to the original weighing plate (used in the calibration of the scale), the correction of measurement errors will be only in the range of the dispensed volume. The same occurs in the calculation of measurement uncertainty.

The improvement of measurements was a key factor for results with less uncertainty. Even though these are small volumes, knowledge and metrological traceability in this range are important due to their use in different segments and the importance of having valid results.

References

1. ISO 8655-2, Piston-operated volumetric apparatus – Part 2: Piston pipettes, 2nd ed., Geneva, International Organization for Standardization, 2022.
2. ISO 8655-6, Piston-operated volumetric apparatus – Part 6: Gravimetric methods for the determination of measurement error, 2nd ed., Geneva, International Organization for Standardization, 2022.
3. ISO 8655-9, Piston-operated volumetric apparatus – Part 9: Manually operated precision laboratory syringes, 1st ed., Geneva, International Organization for Standardization, 2022.
4. OIML R111-1, Weights of classes E1, E2, F1, F2, M1, M1–2, M2, M2–3 and M3 - Part 1: Metrological and technical requirements, 2004
5. Batista, E.; Godinho, I.; Rodrigues, G.; Schork, S. - Pilot study on calibration of micropipettes using the photometric method, Journal of Physics: Conference Series, 2018
6. Batista, E.; Pinto, L.; Filipe, E.; van der Veen, A. Calibration of micropipettes - Test methods and uncertainty analysis. Measurement, 40, 338-342. 2007
7. Barbosa, A. P. F.; Mendonça, A. J. S. M.; Baldner, F. O.; Sereno, H. R.; Lima, L. S.; Sampaio, L. S. Avaliação da influência da utilização da função "tara" em balanças e seus efeitos na calibração de vidrarias de laboratório, II CIMMEC, 1-5. Set/2011
8. EURAMET, Guidelines on the determination of uncertainty in gravimetric volume calibration – CG-19, Version 3.0, Sep/2018
9. Instituto Nacional de Metrologia, Qualidade e Tecnologia - INMETRO, Avaliação de dados de medição: Guia para a expressão de incerteza de medição – GUM 2008, 2012
10. Sampaio, L. S.; Sampaio, B. S.; Tarelho, L. V. G.; Avelino, M. R.; Mendonça, A. J.M.; Soares, D. Calibração de volume em escala nanométrica – Escolha da correta balança, Enqualab, 1-6. 2019
11. Ettre, L.S. **Fifty years of gas chromatography - The pioneers I knew, Part II**, *LCGC North America*, VOL 20, Nº 5, 452-462. May/2002