# The Uncertainty of Volume Calibration using A&D Pipette Accuracy Testers (Considerations of the Gravimetric Method and its Measurement Errors)

## 1. Volume Measurement by the Gravimetric Method

For calibration of capacity meters such as pipettes, ISO8655-6 recommends the gravimetric method, in which the mass value of the distilled water dispensed from a capacity meter is measured with a balance and then converted to a dispensed quantity (volumetric value). No method can measure directly the physical quantity of a minute volume. Therefore, the most common and precise method is to measure the mass value of distilled water, whose physical properties are known, using a balance and then convert the mass to a volumetric value.

Conversion from a mass value to a volumetric value involves the temperature of the distilled water and the barometric pressure as parameters. However, the variation of the measurement results due to barometric fluctuation is negligible, and in practice, it will suffice to set and use a representative value (fixed value) of the location of measurement. Consequently, the equipment to be controlled at the time of volume calibration will be the balance and the thermometer.

What follows is concrete consideration of the uncertainty of volume calibration.

## 2. Uncertainty Components in Volume Calibration by Gravimetric Method

In the gravimetric method, the mass value of the distilled water dispensed from a capacity meter such as a pipette is measured using a balance, and the measured mass value is then converted to a volumetric value. Therefore, the uncertainty of volume calibration can be divided into "2-1 Components concerning the mass measurement using a balance," and "2-2 Components concerning the mass-to-volume conversion."

### 2-1 Components Concerning the Mass Measurement using a Balance

Errors in the mass measurement lead directly to errors after the mass-to-volume conversion. There are errors due to the balance and errors due to the measurement method.

Errors due to the balance will be considered from the product specifications (3-1 Uncertainty based on the performance of the balance).

As for errors due to the measurement method, it is necessary to consider the evaporation of the distilled water during the mass measurement (3-2 Uncertainty based on evaporation).

### 2-2 Components Concerning the Mass-to-Volume Conversion

The density of distilled water is approximately 1 g/mL but varies depending on the temperature (water temperature). Meanwhile, in order to measure the mass of an object precisely, it is necessary to correct for buoyancy since the balance is calibrated using a weight (density 8000 $kg/m^3$). Therefore, these factors (density variation of distilled water due to temperature change and correction for buoyancy) must be considered when converting the measured mass value of the distilled water to a volumetric value.

The conversion factor used to calculate the volume of distilled water from its mass is called the

Z factor (Z correction factor). The volumetric value can be obtained by multiplying the measured mass value with the Z factor.

$$V = m \times Z$$

V : Volume
m : Mass
Z : Z factor

The ISO8655-6 publishes a table for determining the Z factor that has the temperature (water temperature) and the barometric pressure as the parameters (see Table 1 in Exhibit 1). Uncertainty concerning the mass-to-volume conversion relates to uncertainty concerning the temperature (water temperature) measurement and the barometric pressure measurement (3-3 Uncertainty based on the Temperature (water temperature) and Barometric Pressure Measurements).

A&D's pipette accuracy testers (AD-4212B-PT, AD-4212A-PT, and FX-300i-PT) include WinCT-Pipette, special software that automatically calculates the Z factor according to ISO8655-6 and converts mass values into volumetric values using the liquid temperature and barometric pressure previously entered.

### 2-3 Proficiency of Operators

Dispensed volumes of capacity meters as typified by pipettes are known to be influenced by operator skill. Therefore, a dispensed volume depends both on the performance of the capacity meter itself and the level of operator skill. Operator skill is a significant uncertainty component in volume calibration.

## 3. Concrete Consideration of Uncertainty

Uncertainty when performing volume calibration with the following products will be discussed.

| Pipette Accuracy Tester | Calibration Volume |
|---|---|
| AD-4212B-PT | 20 µL |
| AD-4212A-PT | 200 µL |
| FX-300i-PT | 1000 µL |

### 3-1 Uncertainty based on the Performance of the Balance

Repeatability, linearity, rounding error, and sensitivity drift are performance factors of the balance that affect the volume measurement. It is presumed that the balance is properly calibrated at the time of volume measurement.

The following examples of uncertainty arising from the performance of balances were calculated from the specifications of the balances used for the pipette accuracy testers.

1) AD-4212B-PT (Measurement of 20 µL)

| Balance specification | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|
| Repeatability (Standard deviation) | 0.05 mg | Normal | 50 µg | 0.050000 µL |
| Linearity | ±0.05 mg | Rectangular | 28.868 µg | 0.028868 µL |
| Resolution | 0.01 mg | Rectangular | 5.774 µg | 0.005774 µL |
| Sensitivity drift*1 | ±2 ppm/°C | Rectangular | 0.012 µg | 0.000012 µL |

2) AD-4212A-PT (Measurement of 200 µL)

| Balance specification | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|
| Repeatability (Standard deviation) | 0.15 mg | Normal | 150 µg | 0.150000 µL |
| Linearity | ±0.3 mg | Rectangular | 173.205 µg | 0.173205 µL |
| Resolution | 0.1 mg | Rectangular | 57.735 µg | 0.057735 µL |
| Sensitivity drift*1 | ±2 ppm/°C | Rectangular | 0.115 µg | 0.000115 µL |

3) FX-300i-PT (Measurement of 1000 µL)

| Balance specification | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|
| Repeatability (Standard deviation) | 1 mg | Normal | 1000 µg | 1.000000 µL |
| Linearity | ±2 mg | Rectangular | 1154.701 µg | 1.154701 µL |
| Resolution | 1 mg | Rectangular | 577.350 µg | 0.577350 µL |
| Sensitivity drift*1 | ±2 ppm/°C | Rectangular | 0.577 µg | 0.000577 µL |

*1 Uncertainty was calculated with an load equivalent to each calibration volume, with the temperature variation at the time of measurement being considered 1 °C.

Note) Eccentric loading error is not considered here as the sample cup is small and the load is applied on the center of the weighing pan.

## 3-2 Uncertainty based on Evaporation

Once distilled water is dispensed from a capacity meter such as a pipette into the sample cup set on the balance, a certain amount of evaporation will take place before the mass value of the distilled water is determined by the balance. This evaporation amount will be a component of uncertainty. Using the evaporation trap that comes with A&D's pipette accuracy testers, it is possible to keep the evaporation under approx. 0.07 mg/min*2, with the ambient humidity being 50%. If the measurement takes 15 seconds, the amount of evaporation will only be around 0.018 mg (=0.018 µL). For measured volumes of 20 µL and 200 µL, this amount accounts only for 0.09% and 0.009% respectively.

The following is an example of uncertainty based on evaporation.

| | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|
| Evaporation amount | 0.018mg | Rectangular | 10.392 µg | 0.010392 µL |

*2 The amount of evaporation will be approx. 0.3 mg/min if the evaporation trap is not used. If the measurement takes 15 seconds, the amount of evaporation will be around 0.075 µL. For measured volumes of 20 µL and 200 µL, this amount accounts for 0.38% and 0.0375% respectively.

### 3-3 Uncertainty based on the Temperature (Water Temperature) and Barometric Pressure Measurements

The density variation of distilled water due to water temperature is approx. 0.02%/ ˚C between 15 ˚C and 30 ˚C. Therefore, when the error of temperature measurement of distilled water is 1 ˚C, it will be an error of approx. 0.02% after conversion to volume.

The influence of barometric pressure on the conversion to volume will be as minute as 0.01% per pressure change of 100 hPa between 850 hPa and 1050 hPa. The pressure change at one location is normally ±15 hPa. Even though an average (fixed value) is used, the pressure fluctuation can be locked in easily between ±30 hPa, whose influence on the conversion to volume is within ±0.003%.

These influence rates of temperature (water temperature) and barometric pressure on the mass-to-volume conversion can be found readily in the Z-factor table shown in ISO8655-6 (see Table 1 in Appendix 1).

Below are examples of uncertainty based on the specifications of the thermometer provided with A&D's pipette accuracy testers (accuracy: ±1.0 ˚C between 0 ˚C and 60 ˚C, resolution: 0.1 ˚C) under the following suppositional measurement conditions:

- Temperature change during the water temperature measurement is within 1.0 ˚C.
- Error in the barometric pressure measurement is within ±30 hPa.

1) AD-4212B-PT (Measurement of 20 µL)

| Item | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|
| Thermometer: measurement accuracy | ±1.0 ˚C | Rectangular | 0.577 ˚C | 0.002309 µL |
| Thermometer: resolution | 0.1 ˚C | Rectangular | 0.029 ˚C | 0.000115 µL |
| Water temperature change during measurement | 1.0 ˚C | Rectangular | 0.289 ˚C | 0.001155 µL |
| Barometric pressure measurement: accuracy | ±30 hPa | Rectangular | 17.32 hPa | 0.000346 µL |

2) AD-4212A-PT (Measurement of 200 µL)

| Item | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Thermometer: measurement accuracy | ±1.0 ˚C | Rectangular | 0.577 ˚C | 0.023094 µL |
| Thermometer: resolution | 0.1 ˚C | Rectangular | 0.029 ˚C | 0.001155 µL |
| Water temperature change during measurement | 1.0 ˚C | Rectangular | 0.289 ˚C | 0.011547 µL |
| Barometric pressure measurement: accuracy | ±30 hPa | Rectangular | 17.32 hPa | 0.003464 µL |

3) FX-300i-PT (Measurement of 1000 µL)

| Item | Value | Probability distribution | Standard uncertainty | Standard uncertainty of volume calculation |
|---|---|---|---|---|
| Thermometer: measurement accuracy | ±1.0 ˚C | Rectangular | 0.577 ˚C | 0.115470 µL |
| Thermometer: resolution | 0.1 ˚C | Rectangular | 0.029 ˚C | 0.005774 µL |
| Water temperature change during measurement | 1.0 ˚C | Rectangular | 0.289 ˚C | 0.057735 µL |
| Barometric pressure measurement: accuracy | ±30 hPa | Rectangular | 17.32 hPa | 0.017321 µL |

## 3. Summary of Uncertainty of the Pipette Accuracy Testers

From what has been discussed above, uncertainty in volume calibration using A&D's pipette accuracy testers can be calculated as follows:

1) AD-4212B-PT (Measurement of 20 µL)

| Category | Item | Standard uncertainty of volume calculation | Variance | Expanded uncertainty ($k$ = 2) |
|---|---|---|---|---|
| Balance | Repeatability | 0.050000 µL | 2.50e-03 (µL)$^2$ | 0.12 µL |
| | Linearity | 0.028868 µL | 8.33e-04 (µL)$^2$ | |
| | Resolution | 0.005774 µL | 3.33e-05 (µL)$^2$ | |
| | Sensitivity drift | 0.000012 µL | 1.33e-10 (µL)$^2$ | |
| Evaporation | Evaporation amount | 0.010392 µL | 1.08e-04 (µL)$^2$ | |
| Water temperature (thermometer) | Measurement accuracy | 0.002309 µL | 5.33e-06 (µL)$^2$ | |
| | Resolution | 0.000115 µL | 1.33e-08 (µL)$^2$ | |
| | Water temperature change during measurement | 0.001155 µL | 1.33e-06 (µL)$^2$ | |
| Barometric pressure | Measurement accuracy | 0.000346 µL | 1.20e-07 (µL)$^2$ | |

2) AD-4212A-PT (Measurement of 200 µL)

| Category | Item | Standard uncertainty of volume calculation | Variance | Expanded uncertainty ($k$ = 2) |
|---|---|---|---|---|
| Balance | Repeatability | 0.150000 µL | 2.25e-02 (µL)$^2$ | 0.48 µL |
| | Linearity | 0.173205 µL | 3.00e-02 (µL)$^2$ | |
| | Resolution | 0.057735 µL | 3.33e-03 (µL)$^2$ | |
| | Sensitivity drift | 0.000115 µL | 1.33e-08 (µL)$^2$ | |
| Evaporation | Evaporation amount | 0.010392 µL | 1.08e-04 (µL)$^2$ | |
| Water temperature (thermometer) | Measurement accuracy | 0.023094 µL | 5.33e-04 (µL)$^2$ | |
| | Resolution | 0.001155 µL | 1.33e-06 (µL)$^2$ | |

| | | | | |
|---|---|---|---|---|
| | Water temperature change during measurement | 0.011547 μL | 1.33e-04 $(\mu L)^2$ | |
| Barometric pressure | Measurement accuracy | 0.003464 μL | 1.20e-05 $(\mu L)^2$ | |

3) FX-300i-PT (Measurement of 1000 μL)

| Category | Item | Standard uncertainty of volume calculation | Variance | Expanded uncertainty ($k$ = 2) |
|---|---|---|---|---|
| Balance | Repeatability | 1.000000 μL | 1.00e+00 $(\mu L)^2$ | 3.3 μL |
| | Linearity | 1.154701 μL | 1.33e+00 $(\mu L)^2$ | |
| | Resolution | 0.577350 μL | 3.33e-01 $(\mu L)^2$ | |
| | Sensitivity drift | 0.000577 μL | 3.33e-07 $(\mu L)^2$ | |
| Evaporation | Evaporation amount | 0.010392 μL | 1.08e-04 $(\mu L)^2$ | |
| Water temperature (thermometer) | Measurement accuracy | 0.115470 μL | 1.33e-02 $(\mu L)^2$ | |
| | Resolution | 0.005774 μL | 3.33e-05 $(\mu L)^2$ | |
| | Water temperature change during measurement | 0.057735 μL | 3.33e-03 $(\mu L)^2$ | |
| Barometric pressure | Measurement accuracy | 0.017321 μL | 3.00e-04 $(\mu L)^2$ | |

Please note that the following conditions are presumed:

1) Pipette accuracy testers are operating correctly in favorable environments.
2) Balances have been properly calibrated and adjusted at the time of volume measurement.
3) The ambient humidity is 50%RH and the evaporation trap is used.
4) The change in ambient temperature and water temperature during the volume calibration is within 1 ˚C.
5) The measurement error of barometric pressure is within ±30 hPa.

Note) The uncertainty in volume calibration is influenced by environmental conditions. For purposes of accuracy, it is therefore necessary to estimate the uncertainty of actual devices in the environment in which calibration will be performed. The uncertainties of calibration listed above are not guaranteed to be the uncertainties that will be estimated for actual devices.

End

(Appendix 1)

[Table 1] Z Factor Matrix

| Temp. | Barometric pressure (kPa) | | | | | | |
|---|---|---|---|---|---|---|---|
| (˚C) | 80 | 85 | 90 | 95 | 100 | 101.3 | 105 |
| 15.0 | 1.0017 | 1.0018 | 1.0019 | 1.0019 | 1.0020 | 1.0020 | 1.0020 |
| 15.5 | 1.0018 | 1.0019 | 1.0019 | 1.0020 | 1.0020 | 1.0020 | 1.0021 |
| 16.0 | 1.0019 | 1.0020 | 1.0020 | 1.0021 | 1.0021 | 1.0021 | 1.0022 |
| 16.5 | 1.0020 | 1.0020 | 1.0021 | 1.0021 | 1.0022 | 1.0022 | 1.0022 |
| 17.0 | 1.0021 | 1.0021 | 1.0022 | 1.0022 | 1.0023 | 1.0023 | 1.0023 |
| 17.0 | 1.0021 | 1.0021 | 1.0022 | 1.0022 | 1.0023 | 1.0023 | 1.0023 |
| 17.0 | 1.0021 | 1.0021 | 1.0022 | 1.0022 | 1.0023 | 1.0023 | 1.0023 |
| 17.0 | 1.0021 | 1.0021 | 1.0022 | 1.0022 | 1.0023 | 1.0023 | 1.0023 |
| 17.5 | 1.0022 | 1.0022 | 1.0023 | 1.0023 | 1.0024 | 1.0024 | 1.0024 |
| 18.0 | 1.0022 | 1.0023 | 1.0023 | 1.0024 | 1.0025 | 1.0025 | 1.0025 |
| 18.5 | 1.0023 | 1.0024 | 1.0024 | 1.0025 | 1.0025 | 1.0026 | 1.0026 |
| 19.0 | 1.0024 | 1.0025 | 1.0025 | 1.0026 | 1.0026 | 1.0027 | 1.0027 |
| 19.5 | 1.0025 | 1.0026 | 1.0026 | 1.0027 | 1.0027 | 1.0028 | 1.0028 |
| 20.0 | 1.0026 | 1.0027 | 1.0027 | 1.0028 | 1.0028 | 1.0029 | 1.0029 |
| 20.5 | 1.0027 | 1.0028 | 1.0028 | 1.0029 | 1.0029 | 1.0030 | 1.0030 |
| 21.0 | 1.0028 | 1.0029 | 1.0029 | 1.0030 | 1.0031 | 1.0031 | 1.0031 |
| 21.5 | 1.0030 | 1.0030 | 1.0031 | 1.0031 | 1.0032 | 1.0032 | 1.0032 |
| 22.0 | 1.0031 | 1.0031 | 1.0032 | 1.0032 | 1.0033 | 1.0033 | 1.0033 |
| 22.5 | 1.0032 | 1.0032 | 1.0033 | 1.0033 | 1.0034 | 1.0034 | 1.0034 |
| 23.0 | 1.0033 | 1.0033 | 1.0034 | 1.0034 | 1.0035 | 1.0035 | 1.0036 |
| 23.5 | 1.0034 | 1.0035 | 1.0035 | 1.0036 | 1.0036 | 1.0036 | 1.0037 |
| 24.0 | 1.0035 | 1.0036 | 1.0036 | 1.0037 | 1.0037 | 1.0038 | 1.0038 |
| 24.5 | 1.0037 | 1.0037 | 1.0038 | 1.0038 | 1.0039 | 1.0039 | 1.0039 |
| 25.0 | 1.0038 | 1.0038 | 1.0039 | 1.0039 | 1.0040 | 1.0040 | 1.0040 |
| 25.5 | 1.0039 | 1.0040 | 1.0040 | 1.0041 | 1.0041 | 1.0041 | 1.0042 |
| 26.0 | 1.0040 | 1.0041 | 1.0041 | 1.0042 | 1.0042 | 1.0043 | 1.0043 |
| 26.5 | 1.0042 | 1.0042 | 1.0043 | 1.0043 | 1.0044 | 1.0044 | 1.0044 |
| 27.0 | 1.0043 | 1.0044 | 1.0044 | 1.0045 | 1.0045 | 1.0045 | 1.0046 |
| 27.5 | 1.0045 | 1.0045 | 1.0046 | 1.0046 | 1.0047 | 1.0047 | 1.0047 |
| 28.0 | 1.0046 | 1.0046 | 1.0047 | 1.0047 | 1.0048 | 1.0048 | 1.0048 |
| 28.5 | 1.0047 | 1.0048 | 1.0048 | 1.0049 | 1.0049 | 1.0050 | 1.0050 |
| 29.0 | 1.0049 | 1.0049 | 1.0050 | 1.0050 | 1.0051 | 1.0051 | 1.0051 |
| 29.5 | 1.0050 | 1.0051 | 1.0051 | 1.0052 | 1.0052 | 1.0052 | 1.0053 |
| 30.0 | 1.0052 | 1.0052 | 1.0053 | 1.0053 | 1.0054 | 1.0054 | 1.0054 |

Note) The calculation method of the Z factor can be found in ISO/TR20461:2000 (see Document 1 in Appendix 2).

(Appendix 2)

[Document 1] Calculating the Z Factor

*1 The calculation method of the Z factor can be found in ISO/TR20461:2000.

$$Z = \frac{1}{\rho_b} \times \frac{\rho_b - \rho_a}{\rho_w - \rho_a}$$

| | |
|---|---|
| $Z$ | Z factor |
| $\rho_w$ | Density of the distilled water |
| $\rho_a$ | Density of air |
| $\rho_b$ | Density of the weight used for calibrating the balance (8000 kg/m3 in general) |

* Please refer to ISO/TR20461:2000 for how to calculate the air density.