



Consulting Analytical Chemists and Geochemists

ACCURACY, LINEARITY IN CALIBRATION, BEST
CALIBRATION PRACTICE AND PREPARATION OF
MASS / MASS CALIBRATION STANDARDS AND QC
STANDARDS AND ESTIMATION OF THEIR
MEASUREMENT UNCERTAINTY

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Application Note: 33

1. Calibration Best Practice

To minimise the measurement uncertainty on test sample concentrations predicted from linear calibrations, the following needs to be applied:

- ***Maximise the number of solution calibration standards***
Ideally 6 to 8 calibration standards should be used, with at least a minimum of 6.
- ***Ensure equal spacing of calibration standards concentrations***
The standard concentrations need to be prepared so that they are evenly spaced, e.g., 0, 2, 4, 6, 8 and 10 ppm
- ***Maximise the sensitivity of analytical method***
- ***Minimise the distance between the test sample absorbance measurement and the centroid absorbance value***
It is desirable to measure the test sample solutions within close proximity of the centroid of the regression calibration. The centroid is the point on the calibration line at \bar{x} , \bar{y} . In other words, the centroid is the mean of the calibration standard concentrations and the mean of the absorbance values for these calibration standards. The smallest measurement uncertainty exists at the centroid.
- ***Include a matrix blank as part of the calibration***
- ***Verification of linearity in the regression***
Verify if the regression data best fit a linear model using the significance of the quadratic term test.

2. Validation of Accuracy

According to ERM (2005); Eurolab (2007); Abzalov (2011) and Carr (2011), the validation of accuracy for a given mean and certified value requires the inclusion of the measurement uncertainty of the CRM in a t-test for statistical significance. The classical Student's t-test as shown in [2], does not take into account the measurement uncertainty of the CRM. To compensate for this, Eurolab Technical Report No.1/2007 recommends equation [3] for the validation of CRMs with stated measurement uncertainties.

$$t_{calc} = \frac{|\bar{x} - \mu|}{\frac{s}{\sqrt{n}}} \quad [2]$$

$$t_{calc} = \frac{|\bar{x} - \mu|}{\sqrt{(u_{\mu})^2 + \frac{s^2}{n}}} \quad [3]$$

Where, t_{calc} is the calculated t-statistic, \bar{x} the mean of n replicates with a standard deviation of s for a CRM of μ certified value. The standard uncertainty u is the stated expanded uncertainty (U) of the CRM divided by the coverage factor (k) as stated on the certificate of analysis. Note that the $| \quad |$ bars indicate that the absolute value between the mean and the certified value is to be used, *i.e.* ignore the sign.

In validation of accuracy, the *hypothesis question* is:

Is the difference between the observed mean and the certified value statistically significant at a level of confidence of 95%? Alternatively put, is there sufficient evidence to conclude that the data *i.e.* replicates generated, are inaccurate?

The relevant hypotheses are:

Null hypothesis:

H₀: Mean = Certified value of CRM with stated measurement uncertainty. The acceptance of H₀ means that accuracy is demonstrated; *i.e.* insufficient evidence to reject H₀;

Alternate hypothesis:

H₁: Mean \neq Certified value of CRM with stated measurement uncertainty. The acceptance of H₁ means that accuracy is not demonstrated, *i.e.* there is sufficient evidence to accept H₁;

Decision rules

If $p \geq 0.05$, accept H₀

If $p < 0.05$, reject H₀ in favour of H₁

3. Evaluation of linearity: Significance of Polynomial Test

Non-random distributions of residual data are indicators of either heteroscedastic behaviour or significant curvature in the calibration data whereas random distribution can be indicative of data best fitting a straight-line function. Decisions based on visual examination of residual data are at best subjective. Therefore, in order to verify calibration linearity, a polynomial test for significance is done by applying the p -value decision rules to the cx^2 term of a polynomial fit for the calibration data.

Calibration data that best fit a straight line gives a p -value greater than 0.05 for the cx^2 term. With the polynomial evaluation of linearity, the significance of higher order terms in the regression will show if the regression data best fits a 1st or higher order polynomial. Data that better fits a 1st order polynomial should be accepted as the model to use for the prediction of an element/oxide in a test sample.

The procedure is to fit the regression data to a 2nd order polynomial as shown in Equation [4] and then test the x^2 term for statistical significance (Kroll & Emancipator, 1993; Thompson & Lowthian, 2011).

$$y = cx^2 + bx + a \quad [4]$$

The following hypotheses and decision rules are observed in the polynomial evaluation of linearity test:

Null Hypothesis:

H₀: $x^2=0$ (no evidence of curvature in the regression data).

Alternate Hypothesis:

H₁: $x^2 \neq 0$ (evidence of curvature in the regression data).

Decision rules

If $p \geq 0.05$, then x^2 is not significant and the data best fits a linear function.

If $p < 0.05$, then x^2 is significant and curvature is evident.

4. Preparation of ICP Calibration Standards

A gravimetric method in which a standard solution is prepared based on solvent mass to standard solution mass is recommended. The gravimetric method is inherently more accurate than the volumetric method because the temperature of the solvent can be ignored, and the mass of solvent and standard solution are measured using a balance. In addition, the amount of solvent contained by a volumetric flask is a function of temperature, whereas the mass of the solvent is not affected by temperature.

Working gravimetrically, does not require working with volumetric glassware. One only needs to add an approximate volume and you work with the numbers you have. However, these numbers must be recorded throughout the procedure. For all applications you do not even need to weigh to a precise target - you get within 10% (say) of the target and record the actual weight, then enter all the actual weights (actual weight of solvent, actual weight of solute for stock standard, actual weight of diluent, actual weight of stock added) into a spreadsheet and calculate actual mass fractions. Take actual weights of samples, dissolved in actual weights of solvent. If standards and samples are all done gravimetrically then volumes never enter into the calculation.

4.1 Preparation of Calibration Standards

Volumetric dilutions *e.g.*, 10 ml dilution to 100 ml volumetric flask, do carry uncertainties in volume calibration and variations in density. There are too many possible pipetting errors to risk a volumetric transfer without checking the accuracy by weighing the aliquot. However, for convenience, a procedure for a mass volumetric preparation that will minimise the major sources of uncertainty is discussed here. A mass-mass, or gravimetric method is also described below, *i.e.*, the concentration of the standard is prepared as a mass to mass value, $\mu\text{g/g}$ (equivalent to ppm).

4.2 Preparation of Standard Solutions by Mass/Volume

Standard solutions are prepared by transferring an aliquot of the solution standard to an empty, dry polyethylene bottle and then weighing the bottle. The solution is then transferred to a class A volumetric flask and the polyethylene bottle reweighed to determine the exact mass of standard reference material solution transferred. The solution in the flask is then diluted to 99 % of the volume of the flask using an appropriate dilute acid, mixed thoroughly, and the

remaining few drops needed to dilute to exact volume, carefully added. The concentration (in mg/ml) of the resulting standard solution can then be calculated by multiplying the mass (in g) of the standard solution amount by the standard's certified value (in mg/g) and dividing by the calibrated volume (in ml) of the flask used for dilution. Thus, no correction for density is needed, and although the concentration of the resulting standard solution may be an uneven fraction of the original standard solution concentration, it will be known as accurately as a volumetric dilution permits.

Example:

0.5034 g of reference standard solution of concentration 1000 mg/g is transferred to a 500 ml A-grade volumetric flask is. The concentration in mg/ml is:

$$mg/ml = \frac{0.5034 \text{ g} \times 1000 \text{ mg/g}}{1000 \text{ ml}}$$

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$$concentration = 0.5034 \text{ mg/ml}$$

Converting to ppm: mg/ml multiplied by 1000 = mg/l = ppm. The measurement uncertainty on 0.5034 mg/ml (503.4 mg/l or ppm) can be found by calculating the combined uncertainties of the balance, volumetric flask and stated concentration as from the certificate of analysis of the stock solution.

4.3 Preparation of Standard Solutions by Mass (Gravimetric Method)

Each standard solution should be prepared by transferring an aliquot of the solution standard to an empty, dry, pre-weighed polyethylene bottle (or glass) and then reweighing the bottle. An appropriate dilute acid must be added by mass to bring the solution to the approximate desired dilution. The dilution need not be exact since the mass of the empty bottle, mass of the bottle plus solution standard aliquot, and the final diluted mass of the solution will permit calculation of the exact concentration of the working solution. The working standard solution concentration will be in mg/kg units and is easily calculated provided the density of the standard solution is known.

An example is given here of the procedure to prepare standards by gravimetric means:

A primary stock solution purchased from a standards supplier of calcium at 1000 ug/ml has a density of 1.016 g/ml. Since we know the density, the parts-per-million ($\mu\text{g/g}$) is calculated as:

$$\frac{1000\mu\text{g/ml}}{1.016\text{g/ml}} = 984.25\text{ppm or } 984.25\mu\text{g/g}$$

From this concentration it is then just a simple matter of diluting to the desired concentration by weighing the stock and intermediate standards into a known mass of diluent (see Table 1). The equipment to be used includes: an analytical balance accurate to 0.1 mg, Pasteur pipettes (or similar) and several clean polyethylene plastic bottles.

To calculate the actual concentration of a calibration standard:

$$\frac{\text{mass (g) of } 10\text{ppb standard} \times \text{concentration of } 10\text{ppb standard}}{\text{mass of final dilution volume (g)}}$$

An example:

$$\frac{6.0153(\text{g}) \text{ of } 9.8425 \text{ ng/g}}{100.000\text{g}} = 0.5921\text{ppb}$$

Calculations of Concentration for Dilutions

One method of preparing a series of calibration standard solutions is to prepare a secondary stock solution from a primary stock solution, from which it a series of dilutions for a range of concentrations can be prepared. The mass of the diluent and that of the added standard solution is used to calculate the concentrations of the dilutions. The stock solution concentration should be the highest concentration you wish to measure.

Preparation of dilutions:

1. Prepare a stock solution using either method listed above.
2. Use clean glassware or plasticware for dilutions.
3. To reach a target concentration (c_{target}) with a solution of volume v_{solution} , the volume of stock, v_{stock} required is:

$$v_{stock} = v_{solution} \frac{C_{target}}{C_{stock}}$$

4. Weigh the empty flask.
5. Add the target volume of stock solution using a clean pipette or syringe.
6. Weigh the flask and stock solution.
7. Subtract the flask and stock solution mass from the empty flask mass, to obtain the mass of stock added, m_{stock} .
8. Add enough solvent to reach desired solution volume, that is $v_{solution} - v_{stock}$, then weigh the flask and dilute the solution.
9. Subtract to obtain total mass of solution, $m_{solution}$
10. Swirl the flask to ensure good mixing.

Calculation of Concentration:

The concentration of a dilution is calculated using:

$$C_{dilute} = C_{stock} \frac{m_{stock}}{m_{solvent}}$$

where C_{dilute} is the concentration of the diluted solution.

Example:

A target concentration of approximately 50 $\mu\text{g/g}$ (ppm) and of 100 ml is to be prepared as a secondary stock solution for further dilution to make a series of calibration standards. The density of the primary stock standard solution is 1.016 g/ml with a given concentration of 1000 $\mu\text{g/ml}$. Therefore, the concentration of the primary stock solution in $\mu\text{g/g}$ is:

$$\frac{1000 \mu\text{g/ml}}{1.016\text{g/ml}} = 984.25\mu\text{g/g} \text{ (984.25ppm)}$$

Calculating the volume of stock solution required to prepare an approximately 50 $\mu\text{g/g}$ secondary stock solution:

$$v_{primary\ stock} = v_{secondary\ standard} \frac{C_{target}}{C_{stock}}$$

$$v_{primary\ stock} = 100\text{ ml} \cdot \frac{50\ \mu\text{g/g}}{1000\ \mu\text{g/g}}$$

$$v_{primary\ stock} = 100\text{ ml} \cdot \frac{50\ \frac{\mu\text{g}}{\text{g}}}{1000\ \frac{\mu\text{g}}{\text{g}}} = 5\text{ ml}$$

Using a micropipette or glass pipette, approximately 5 ml of stock solution is added to a clean tared plastic container. The mass of the 5 ml stock solution transferred is weighed as 5.0235 grams. The plastic container is again tared and approximately 100 ml of diluent is added, and its mass is recorded as 100.102 grams. The concentration of the secondary standard solution is found as:

$$c_{dilute} = 984.25 \mu\text{g/g} \cdot \frac{5.0235 \text{ g}}{100.102 \text{ g}}$$

$$c_{dilute} = 984.25 \mu\text{g/g} \cdot \frac{5.0235 \text{ g}}{100.102 \text{ g}}$$

$$c_{dilute} = 49.394 \mu\text{g/g}$$

Therefore, the concentration of the secondary stock solution is 49.394 $\mu\text{g/g}$.

To prepare standards covering the desired analytical range, a series of standards are prepared from the 49.394 $\mu\text{g/g}$ secondary stock solution by dilution to an approximate volume of 100 ml. The mass of standard and diluent is recorded for each and the concentration in $\mu\text{g/g}$ is calculated.

Table 1. Example of mass and dilution for preparation of a range of calibration standards of 0 to 50 ppm as mass/mass concentrations.

Target Conc. ($\mu\text{g/g}$)	Approx. vol. (ml) of 50 ($\mu\text{g/g}$) Stock Solution	Mass of 100 ($\mu\text{g/g}$) Secondary Stock (g)	Mass of diluent (g)	Final Conc. ($\mu\text{g/g}$)
0	0	0.000	100.2350	0
10	1	1.0034	100.0125	9.875
20	2	2.0023	100.0234	19.703
30	3	3.0012	99.0145	29.833
40	4	3.9778	99.9023	39.190
50	5	5.0235	100.1020	49.394

4.4 Measurement Uncertainty

An estimation of the uncertainty in the mass/mass concentration of the three independent calibration solutions is required for verification of statistical accuracy over the measurement range. The sources of the uncertainty identified in the preparation of the secondary (independent standards) as well as the calibration standards are:

- Uncertainty in the balance used for weighing
- Uncertainty in the concentration of the primary stock solution
- Uncertainty in the atomic mass of the analyte, i.e., Ca
- Uncertainty in the density

As an example, the measurement uncertainty as an expanded uncertainty for the secondary stock solution is calculated below.

Primary stock solution:

1000 µg/ml ±0.5 mg/l at $k=2$ (95% level of confidence)

Density of stock solution:

There is no quoted measurement uncertainty for the density of 1.016g/ml

The standard uncertainty of the Secondary Stock Solution ($u_{sec.standard}$) (nominal 100 µg/ml), in ug/g is:

$$u_{sec.standard} = C_{primary\ stock} \sqrt{\left(\frac{2u_{mp}}{m_{ps}}\right)^2 + \left(\frac{2u_{md}}{m_{diluent}}\right)^2 + \left(\frac{u_{atomic\ m}}{A\ MASS}\right)^2 + \left(\frac{u_{C\ primary\ stock}}{C_{primary\ stock}}\right)^2}$$

Where,

- $C_{primary\ stock}$ is the concentration of the primary stock solution given as 1000 µg/ml ±0.5 mg/l.
- m_{ps} is the mass of primary stock solution weighed out on an analytical balance of 0.1 mg capability.
- $2u_{mp}$ is the standard uncertainty of the analytical balance used to weigh out the primary stock solution, multiplied by two since the balance is tared and the mass of the solution is measured.
- $2u_{md}$ is the standard uncertainty of the analytical balance used to weigh out the diluent, multiplied by two since the balance is tared and the mass of the solution is measured.
- $m_{diluent}$ is the mass of diluent weighed out on the analytical balance.
- $u_{atomic\ m}$ is the standard uncertainty of the atomic mass of the analyte. In this case, Ca, which according to <https://www.iupac.org/publications/pac/pdf/2006/pdf/7811x2051.pdf> is 40.078(4), g/mole. The (4) represents an uncertainty in the atomic mass, however, it is not clear

whether that stated is a standard or expanded uncertainty, therefore the given uncertainty is divided by the default of $\sqrt{3}$ for a rectangular distribution.

- A_{Mass} is the atomic mass of calcium, which is 40.078 g/mole
(<https://www.iupac.org/publications/pac/pdf/2006/pdf/7811x2051.pdf>)
- $C_{primary\ stock}$ is the concentration of the primary stock solution, i.e., 1000 µg/ml, with $u_{C\ primary\ stock}$ the standard uncertainty of the concentration of the primary stock solution.

Substituting:

$$u_{sec.standard} = 925.93 \mu g/g \sqrt{\left(\frac{2[0.0003g]}{10.0025g}\right)^2 + \left(\frac{2[0.0003g]}{99.9850g}\right)^2 + \left(\frac{0.00023g/mole}{40.078g/mole}\right)^2 + \left(\frac{2.5\mu g/ml}{1000\mu g/ml}\right)^2}$$

$$u_{sec.standard} = (925.93 \mu g/ml) \sqrt{0.0000062536674}$$

$$u_{sec.standard} = 0.2316$$

Since there are three uncertainties quantified, the $t_{critical}$ value for 2 degrees of freedom is 3.182.

Multiplying this by $u_{sec.standard}$ gives the expanded uncertainty:

$$U = 3.182 u_{sec.standard} = 0.737 \mu g/g$$

$$U = \pm 0.74 \mu g/g$$

Therefore, the concentration of the secondary standard stock solution with expanded uncertainty at $k=3.182$ (95% level of confidence) is:

$$925.93 \pm 0.74 \mu g/g$$

Preparing an independent calibration check solution, should be prepared from a different primary stock solution to that which was used to prepare the range of standard calibration solutions.

An example is given below for the preparation of a nominal concentration of 5 ppm as a check standard for the lower end of an existing calibration. Also shown in the estimation of the measurement uncertainty of the nominal 5 ppm concentration. The primary stock standard concentration is given as, $1003 \pm 2 \mu g/ml$, with a density of 1.082 g/ml. If a nominal concentration of 5 ppm is required of 100 ml volume, then:

Using a micropipette or glass pipette, approximately 0.5 ml of stock solution is added to a clean tared plastic container. The mass of the 0.5 ml stock solution transferred is weighed as 0.51005 grams. The plastic container is again tared and approximately 100 ml of diluent is added, and

its mass is recorded as 100.102 grams. The concentration of the secondary standard solution is found as:

$$c_{dilute} = \frac{1003 \mu\text{g/ml}}{1.082 \text{ g/ml}} \cdot \frac{0.51005 \text{ g}}{100.005 \text{ g}}$$

$$c_{dilute} = 926.99 \mu\text{g/g} \cdot \frac{0.51005 \text{ g}}{100.005 \text{ g}}$$

$$c_{dilute} = 4.728 \mu\text{g/g}$$

Therefore, the concentration of the secondary stock solution is 4.728 $\mu\text{g/g}$. The expanded uncertainty is found as:

$$u_{sec.standard} = 926.99 \mu\text{g/g} \sqrt{\left(\frac{2[0.0003 \text{ g}]}{10.0020 \text{ g}}\right)^2 + \left(\frac{2[0.0003 \text{ g}]}{100.0045 \text{ g}}\right)^2 + \left(\frac{0.00023 \text{ g/mole}}{40.078 \text{ g/mole}}\right)^2 + \left(\frac{1 \mu\text{g/ml}}{1003 \mu\text{g/ml}}\right)^2}$$

$$u_{sec.standard} = 0.0073 \mu\text{g/g}$$

Since there are three uncertainties quantified, the $t_{critical}$ value for 2 degrees of freedom is 3.182. Multiplying this by $u_{sec.standard}$ gives the expanded uncertainty:

$$U = 3.182 u_{sec.standard} = 0.023 \mu\text{g/g}$$

$$U = \pm 0.023 \mu\text{g/g}$$

Therefore, the concentration of the independent standard solution with expanded uncertainty at $k=3.182$ (95% level of confidence) is:

$$4.728 \pm 0.023 \mu\text{g/g}$$

The ± 0.023 expanded uncertainty converted to a standard uncertainty (dividing by $k=3.182$) is to be used in the one-sample, two-tailed t-test for the validation of accuracy [equation 3].

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