

## Using Reference Materials for Calibration. Background

*As the first in a series dedicated to calibration, this note explains principles for the use of reference materials in analytical calibration, that is, calibration for the purpose of determining the response behaviour of analytical instruments. Issues addressed include the estimation and use of calibration uncertainty, and requirements on reference materials for calibration. ERM® are perfectly suited for this purpose, because they fulfil the stated requirements.*

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### INTRODUCTION

One of the basic requirements of ISO/IEC 17025 is that all equipment having a significant effect on the accuracy or validity of measurement results provided by a laboratory shall be calibrated before being put into service. To this end, laboratories, especially accredited ones, must have an established calibration programme which ensures that measurements are traceable [1] to the International System of Units (SI) or to other agreed references.

The term “calibration” is currently defined in reference [1] as the

“operation that, under specified conditions, in a first step, establishes a relation between the **quantity values** with measurement uncertainties provided by **measurement standards** and corresponding **indications** with associated **measurement uncertainties** and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication”.

For a chemist in an analytical laboratory this general definition means that calibration determines the relationship between measured instrumental response and amount of the substance to be determined (the analyte) using appropriate calibration materials, and also the calculation used to obtain the result from a new observation. Establishing this relationship is key to establishing traceability to SI units or other appropriate references.

### ANALYTICAL CALIBRATION: FOCUS ON INSTRUMENTAL RESPONSE

Analytical instruments are typically calibrated using one or more calibration materials containing known amounts of the analyte. Most often these are synthetic materials such as calibration solutions, prepared from pure substances, but for special analytical techniques

(e.g. direct analysis of solid samples by SS-AAS or XRF) matrix materials are used for calibration.

### PRINCIPLES OF CALIBRATION: SINGLE-POINT CALIBRATION

#### 1) Calibration

The basic principles of calibration can be illustrated by the simplest case: single-point calibration. This is used when the instrumental response (such as the peak area in a chromatogram) is, perhaps after baseline correction, strictly proportional to the amount of analyte. Then calibration may be carried out at a single level, by replicate analyses of a single calibration material. From the calibration data ( $\bar{y}_{cal}$  is the mean value of response;  $x_{cal}$  is the reference value of analyte amount) the response factor is determined according to

$$F = \frac{\bar{y}_{cal}}{x_{cal}} \quad (1)$$

The response factor is then used to convert instrumental response data  $y_{samp}$  obtained on other samples into analyte amounts  $x_{samp}$  according to

$$x_{samp} = \frac{y_{samp}}{F} \quad (2)$$

Equations (1) and (2) illustrate the two steps in calibration; establishing a relationship between reference value  $x_{cal}$  and response  $y$ , and then using this to derive a calculation to predict values  $x_{samp}$  from new measured values  $y_{samp}$ .

#### 2) Calibration uncertainty

Like any other measurement result the result of a calibration – whether calculated using a factor or a function summarising the calibration data – has an associated uncertainty. This uncertainty has to be included in the uncertainty budget of any result obtained using the calibrated equipment [2,3]. In the case of single-level calibration the uncertainties directly associated with calibration may be expressed as follows:

$$\frac{u_{cal}(x_{smp})}{x_{smp}} = \sqrt{\left(\frac{s(y_{smp})}{y_{smp}}\right)^2 + \left(\frac{s(\bar{y}_{cal})}{\bar{y}_{cal}}\right)^2 + \left(\frac{u(x_{cal})}{x_{cal}}\right)^2} \quad (3)$$

In this expression, all the terms are in the form of relative standard uncertainties.  $u_{cal}(x_{smp})$  is the uncertainty in  $x_{smp}$  arising from calibration. The different terms on the right represent, respectively:

- the uncertainty arising from variation in the response obtained on a sample,
- the uncertainty arising from variation in the response from calibration material,
- the uncertainty associated with the reference value used in calibration.

All of these uncertainty contributions are combined as a root sum of squares.

**Note:** There are usually other sources of uncertainty in a complete measurement. These can often be combined with the calibration uncertainty by simple extension of equation (3). Details can be found in references [2] and [3].

The structure of the uncertainty budget described here can be used for other calibration designs, by combining the following contributions to the standard uncertainty of the result:

- the variability of measurements for the given sample, expressed as a standard deviation;
- the variability of measurements on calibration materials, expressed as a standard deviation;
- the standard uncertainty of the reference values attributed to the calibration materials.

The standard uncertainty reported to the customers additionally requires the analyst to consider the standard uncertainty associated with other effects not covered by the calibration.

## MATRIX EFFECTS

The sample matrix can bias the analyte signal through spectral and non-spectral interference effects. Non-spectral interferences or matrix effects as they are often known, are characterized by changes in signal intensity that are matrix-induced and not related to spectral overlap. Matrix effects can lead to signal

suppression, as well as signal enhancements. An important issue is that matrix effects often depend on the absolute matrix concentration not on the relative concentration of matrix to analyte. Matrix effects can be reduced by simply diluting the sample (if permitted by analyte concentration) or corrected for by certain calibration strategies such as internal standardisation, standard addition, matrix-matched calibration or isotope dilution mass spectrometry. More details on matrix effects and calibration strategies compensating for that can be found in the following literature [4,5].

## REQUIREMENTS FOR CALIBRATION MATERIALS

Calibration materials have to be sufficiently homogeneous and stable so as to ensure that the assigned property values (including uncertainty) are valid for any portion of the calibration sample taken and utilised according to the supplier's specification. In addition, technical requirements for calibration materials are case-dependent and cannot be summarised in a single application note. The only generic requirements that can be addressed here concern the information provided for a calibration material:

- For each reference value (analyte concentration)  $x_{ref}$  the standard uncertainty  $u(x_{ref})$  has to be specified, either directly or by way of specifying an expanded uncertainty  $U(x_{ref})$  with the associated coverage factor  $k$ . In addition a statement of traceability is required (Application Note 3 "Using Reference Materials to Establish Traceability").
- For matrix materials, the matrix needs to be specified in sufficient detail to enable comparison with sample matrices where analyte-matrix interferences may occur.

## EXAMPLES

Examples are explained in Application Note 2b "Using Reference Materials for Calibration. Examples".

- [1] International Vocabulary of Metrology — Basic and General Concepts and Associated Terms, 3<sup>rd</sup> edition (VIM 3) available from <http://www.bipm.org> or as ISO/IEC Guide 99-12:2007
- [2] Hässelbarth W, Guide to the Evaluation of Measurement Uncertainty for Quantitative Test Results, Eurolab Technical Report No. 1/2006, [www.eurolab.org](http://www.eurolab.org)
- [3] Ellison SLR, Williams A, Roesslein M (Eds).: Quantifying Uncertainty in Analytical Measurement 2<sup>nd</sup> Ed. (2000). Eurachem/CITAC Guide, available at [www.eurachem.org](http://www.eurachem.org).
- [4] Thompson M, Ellison SLR, Analytical Methods Committee Report: A review of interference effects and their correction in chemical analysis with special reference to uncertainty, Accred Qual Assur (2005) 10:82–97
- [5] Vogl J, *Calibration strategies and quality assurance*, in Nelms S (ed.) "ICP Mass Spectrometry", Blackwell Publishing Ltd. (2005) 147-181