GENERAL ASPECTS OF TRACE ANALYTICAL METHODS I. METHODS OF CALIBRATION IN TRACE ANALYSIS

Project Leader
GEORGE H. MORRISON
Department of Chemistry, Cornell University, Ithaca, NY 14850, USA

GENERAL ASPECTS OF TRACE ANALYTICAL METHODS
I. METHODS OF CALIBRATION IN TRACE ANALYSIS

Project Leader

GEORGE H. MORRISON

Department of Chemistry, Cornell University, Ithaca, NY 14850, USA

The important role of very small amounts of substances in physical, chemical and biological systems has emerged as methods of analysis have increased in sensitivity. Much of this progress may be attributed to the demands of materials and biological research and to the availability of modern instrumentation. Although a large number of different trace techniques have evolved, they all have a common goal—the measurement of the composition of the species of interest at the parts per million level or lower.

A characteristic feature of most physical or physicochemical determinations is the necessity for finding empirically the value of the intensity factor corresponding to the concentration of a given constituent. The majority of these methods, therefore, require the use of a standard containing a known amount of constituent, which serves as a basis for comparison in the measurements. Thus, calibration is an essential aspect of quantitative trace analysis.

The use of standard samples performs another important function in trace analysis. The ultimate goal in quantitative analysis is accuracy, i.e. the ability to approach the 'true' value. Errors affecting an experimental result may be classified as either systematic or random. The former, occasionally referred to as determinate errors, are due to causes over which the analyst has control, and their undesirable effects on the accuracy of the result can be avoided or corrected. Random errors are not subject to control and are manifested even in the absence of systematic errors, by variations in the result. Although the magnitude of random variations can be reduced by carefully keeping all operations identical, they are never eliminated entirely. The precision of the result thus depends on the random errors. In this situation the distinction between precision and accuracy becomes less apparent, that is the *precision defines the accuracy that can be achieved in the absence of*

---

systematic errors[1]. The accuracy of a trace method can be tested by analyzing standard samples or by comparison with other methods of analysis.

Because of the widespread use of physical and physicochemical methods and the need for higher accuracy, this report reviews the methods of calibration used in trace analysis. It should be noted that errors can arise in the three component steps of an analytical method: sampling, chemical and/or physical pretreatment, and instrumental measurement[2]. Calibration methods must apply to the total analytical procedure. These calibration methods are dependent upon the use of standards. Obviously the concentrations of the standards must be reliably known; and, when chemical interferences are operative, the preparative histories of both samples and standards must be similar or both histories must be reduced to a common state. It is a truism that the accuracy of an analysis depends ultimately on the accuracy of the standards.

CLASSIFICATION OF CALIBRATION METHODS

1. Use of Certified Standards

This type of calibration is especially important for analytical procedures used for complex analyses of large series of similar samples. A set of homogeneous standard samples which cover the whole range of compositions in question must be selected. The preparation of highly reliable standards, particularly solid-state standards, at the trace and ultratrace levels is a singularly difficult task. The lack of trace standards is recognized as a prime limiting factor in the field and is receiving considerable attention from interested parties and agencies in many countries.

It is worth noting that the US National Bureau of Standards has recently made available a number of Standard Reference Materials specifically oriented toward trace analysis. For biological materials they have prepared dried, powdered, orchard leaves [SRM 1571] and bovine liver [SRM 1577]. For geological materials they have prepared glass wafers doped with 61 elements at the 1-p.p.m. [SRM 614/615] and 0.2-p.p.m. [SRM 616/617] level. These certified standard samples are of particular value for calibrating multielement trace methods involving emission spectroscopy, spark source mass spectrometry, neutron activation analysis, and x-ray fluorescence. Because neutron activation analysis has a high sensitivity for the majority of elements, together with an intrinsic high degree of accuracy, the NBS has used this method widely for certification of standard reference materials involving elemental trace analysis[3].

In the area of biomedical standards for use in clinical laboratories, the NBS has recently issued an impressive list of standard reference materials[4]. These include cholesterol [SRM 911], urea [SRM 912], uric acid [SRM 913], creatinine [SRM 914], calcium carbonate [SRM 915], bilirubin [SRM 916], glucose [SRM 917], and potassium chloride [SRM 918].

Table 1 lists a number of agencies and laboratories that provide standards for use in biological, geological, glass and metallurgical analyses. Although the number of certified standard samples is increasing significantly, it is impossible to provide standards for the many different types of materials and analytical problems that the modern analyst is confronted with.

Table 1. Sources of standards

Biological
    Dr H. J. M. Bowen, University of Reading, UK
    International Atomic Energy Agency, Vienna, Austria
    Dr A. L. Kenworthy, Michigan State University, Lansing, Michigan, USA
    Dr M. Merlini, Ispra, Italy
    Tobacco & Health Research Institute, University of Kentucky, Lexington,
      Kentucky, USA
    US National Bureau of Standards

Geological
    Canadian Association for Applied Spectroscopy
    Centre de Recherches Petrographiques et Géochimiques, Nancy, France
    East German Zentrales Geologisches Institut
    Geological Institute of Bulgaria
    Geological Survey of Japan
    Geological Survey of Tanzania
    Mines Branch, Department of Energy, Mines & Resources, Ottawa, Canada
    National Institute of Metallurgy, South Africa
    US Geological Survey, Washington, DC

Glass and Related Standards
    British Chemical Standards
    Centre de Recherches Petrographiques et Géochimiques, Nancy, France
    US National Bureau of Standards

Metallurgical
    The list of government and industrial sources of metallurgical standards in various
countries is too large to include here.

---

2. Use of Independently Analyzed Samples

In the absence of available certified standards for methods involving complex materials, calibration against independent analytical methods is often resorted to. Several determinations should be performed by each method, so that good estimates of the precision of each method may be obtained. If there is a significant difference between the two means obtained, systematic error is probably present in one or both methods. Although the magnitude and the sign of the discrepancy between the two means may indicate the nature of the error, further work must usually be done to discover its source. If there is no significant difference between the two means, there is no reason to suspect the presence of appreciable systematic error in either method.

The success of this approach depends upon the reliability of the comparative method, since it too must have been properly calibrated. In the field of trace element analysis, the use of neutron activation analysis as an independent method is particularly helpful. Since neutron activation analysis is based on the nuclear properties of the constituents of a sample, it is not dependent upon their chemical form, so that synthetic standards may be employed for calibration. Similarly, wet chemical methods and flame spectroscopy can be used when limited numbers of elements are involved, since they can often be calibrated with synthetic standards.

If large numbers of samples of a similar nature are to be analyzed, it may be more practical to choose a comparable sample as an 'in-house' standard and analyze it for the species of interest by a variety of independent methods where applicable. This standard can then be used for future analyses of this type of sample.

3. Standard Addition

In the analysis of complex materials both matrix and interelement effects are the main contributors to systematic errors in many spectrometric methods. This is true if we assume that spectral line interferences are absent. Therefore, the use of certified standards or the independent method of analysis approach on similar materials help to compensate for these complexities.

Another approach that is sometimes used is the method of standard additions, where a small known concentration of the desired species is increasingly added to several samples of the unknown material. The resulting samples as well as an untreated one are then analyzed. The response readings from the particular instrument are then plotted linearly against the added concentrations, and the amount of unknown species present is determined by extrapolating a line to the abscissa. If the line is not linear, often a transformation can be performed such as the conversion of light absorption to absorbance. The best line which can be fitted to the data minimizes the sum of the squares of the vertical distance between data points and the constructed line, referred to as the line of least-squares[5].

The least-squares line can be described by the equation

$$Y = MX + I \qquad (1)$$

where Y is the value predicted (absorbance) by the equation for a given value of X (concentration), M is the slope of the line, and I is the intercept of the line with the ordinate axis. Using data obtained from an analytical determination of treated and untreated samples, the slope of the least-squares line can be calculated

$$\text{Slope, } M = \frac{n\Sigma XY - \Sigma X\Sigma Y}{n\Sigma X^2 - (\Sigma X)^2} \qquad (2)$$

where X refers to the concentration of the standard solution, Y is the linear instrument response reading, and n is the total number of readings made.

Equation 1 is referred to as the regression of Y on X, X being the independent variable and Y the dependent variable. The X values are assumed to be known without error, whereas the Y values are randomly distributed about some mean Y value[6, 7].

The intercept of the line with the ordinate axis is calculated as follows

$$\text{Intercept, } I = \bar{Y} - M\bar{X} \qquad (3)$$

where $\bar{Y}$ is the arithmetic mean of the total Y readings, $\bar{X}$ is the arithmetic mean of the total X values, and M is the slope of the line. An estimate of the precision of the method of standard additions based upon linear least-squares regression has been presented by Larsen et al.[8].

The method is well established and has greater applicability in flame emission and atomic absorption methods and spectrophotometric methods, where the number of components to be determined in a given analysis is limited.

4. Use of Synthetic Standards

Standard samples which have been synthesized from pure substances have had a long history in chemical analysis, but are not too applicable to many trace methods. These synthetic samples can be prepared with reasonable expenditure only for relatively simple analytical problems, where the type of sample and the components to be determined are well characterized. Furthermore, only a few components should be involved. This approach is of value in trace methods that involve a solution step in the procedure such as spectrophotometry, flame spectrometric methods, and electrochemical methods. Preparation of multielement solid standards by powder dilution techniques are impractical and subject to considerable error in addition to ignoring the differences in chemical form of the standard and sample.

In organic analysis, and particularly in biochemical analysis, it may be difficult to obtain the proper starting materials. If traces of contaminants are to be determined in substances of high purity, then it may be impossible to obtain these substances free from any contamination at all.

5. Use of Fundamental Relationships[9]

In limited situations, it is also possible to achieve calibration of measured quantities by deriving the contents to be determined using general principles and known data. Such laws for example include the law of mass action, the Beer–Lambert law, the Boltzmann distribution, etc.

General laws are of course idealized, and are valid only if definite assumptions are correct. In most cases they have only been found and verified under special, very pure experimental conditions. Even in cases where a theoretically derived relationship has been established and has long been proven, its validity has usually been verified by the use of synthetic standards. The use of fundamental relationships as a method of calibration is of some value for new analytical procedures. It provides a first approximation until it is either verified or replaced by calibration with synthetic standards.

CALIBRATION CURVES

In the use of external standards in many methods, the analyst uses a linear calibration curve obtained from measurements made on these standards to estimate the concentration of the unknown. Irving[10] has described a general graphical method for evaluating experimental results that should fit a linear equation. When other than linear relationships are involved, weighted least-squares curve fitting using functional transformations can be employed. Jurs[11] has described transformations from exponential to linear, Gaussian to quadratic, and Cauchy to quadratic. The method is applicable to experimental curve fitting and for the development of calibration curves.

Linning and Mandel[12] have discussed the determination of the precision of an analytical method involving a calibration curve. They emphasize that, because there is always some scatter in the calibration data, the precision of

analysis for an unknown will be poorer than indicated from several repeat determinations of the same sample.

Hubaux and Vos[13] have investigated the influence of the precision of the analytical method, the number of standards, the range of their contents, the various modes of their repartition, and the replication of measurements on the determination of detection limits from linear calibration curves employing a statistical approach.

STABILITY OF CALIBRATION[9-14]

The problem of how long and under what circumstances the calibration values derived for a given analytical procedure remain valid is of importance for the critical assessment of a procedure and of the analytical results produced by it. Stability of calibration involves two aspects:

(a) the sensitivity of the analytical procedure to variations of experimental parameters, and
(b) the extent to which one has control over the parameters to keep the experimental conditions constant or to take into account the influence of changing parameters by suitable measurements and corrections.

Complex analytical procedures are usually susceptible to parameter changes, since it is difficult to reduce all of the sensitivities to variations simultaneously and throughout the whole range. Frequent calibration is mandatory.

CONCLUSION

While an attempt has been made here to enumerate the methods of calibration employed in trace analysis, it is difficult to compare them in any quantitative fashion. To date there has been no study to evaluate the different methods as applied to a given procedure. In view of the many different situations encountered in trace analysis, it is obvious that each calibration method has a role to play in a given circumstance.

In the absence of any direct comparisons of methods of calibration in trace analysis, a study is cited where an evaluation was made of elemental analyses of a given lunar soil sample by a large number of investigators using a variety of methods employing many different techniques such as neutron activation analysis, spark source mass spectrometry, emission spectroscopy, x-ray fluorescence, and various other forms of mass spectrometry[15]. Taking into account such factors as heterogeneity of sample, concentration levels, contamination, etc., the conclusion reached is that there is considerable variability in the performance of different laboratories, regardless of the methods employed. This can only be ascribed to the care with which the analyses were performed and the attention paid to the proper use of calibration.

REFERENCES

[1] G. H. Morrison and R. K. Skogerboe, 'General aspects of trace analysis', in *Trace Analysis: Physical Methods*, p 1. G. H. Morrison, editor. Interscience: New York (1965).

[2] R. K. Skogerboe and G. H. Morrison, 'Trace analysis: essential aspects', in *Treatise on Analytical Chemistry*, Part 1, Vol. IX, p 5836, edited by I. M. Kolthoff and P. J. Elving. Wiley: New York (1971).

[3] G. W. Smith, D. A. Becker, G. J. Lutz, L. A. Currie and J. R. DeVoe, *Anal. Chim. Acta*, **38**, 330 (1967).

[4] W. W. Meinke, *Anal. Chem.* **43**(6), 28A (1971).

[5] C. L. Grant, 'Statistics helps evaluate analytical methods', in *Developments in Applied Spectroscopy*, Vol. VI, p 115. W. K. Baer, A. J. Perkins and E. L. Grove, Eds. Plenum: New York (1968).

[6] J. Mandel and F. J. Linning, *Anal. Chem.* **29**, 743 (1957).

[7] G. W. Snedecor and W. C. Cochran, *Statistical Methods*, 6th ed. Iowa State University Press: Ames, Iowa (1968).

[8] I. L. Larsen, N. A. Hartmann and J. J. Wagner, *Anal. Chem.* **45**, 1511 (1973).

[9] H. Kaiser, 'Foundations for the 'critical discussion of analytical methods', in *Methodicum Chimicum*, Academic Press: New York, in press.

[10] H. M. N. H. Irving, *Analyst*, **93**, 273 (1968).

[11] P. C. Jurs, *Anal. Chem.* **42**, 747 (1970).

[12] F. J. Linning and J. Mandel, *Anal. Chem.* **36**(13), 25A (1964).

[13] A. Hubaux and G. Vos, *Anal. Chem.* **42**, 849 (1970).

[14] H. Kaiser, *Pure Appl. Chem.* **34**, 35 (1973).

[15] G. H. Morrison, *Anal. Chem.* **43**(7), 22A (1971).