1

Calibration Fundamentals

The general understanding of the term "calibration" is far from what applies to the concept in an analytical sense. Leaving aside colloquial connotations, such as calibrating a weapon, the term is generally associated with the adjustment of specific parameters of an object to fixed or desired quantities, and in particular with the adjustment of a specific instrument to perform a correct function. It is, therefore, understood more as a process of instrumental standardization or adjustment. This is reinforced by publicly available nomenclatural sources. For example, in the Cambridge Advanced Learner's Dictionary [1] calibration is defined as " … the process of checking a measuring instrument to see if it is accurate," and in the http://Vocabulary.com online dictionary as "the act of checking or adjusting (by comparison with a standard) the accuracy of a measuring instrument" [2]. Even in a modern textbook in the field of instrumental analysis, you can read: "In analytical chemistry, calibration is defined as the process of assessment and refinement of the accuracy and precision of a method, and particularly the associated measuring equipment…" [3].

The ambiguity of the term "calibration" makes it difficult to understand it properly in a purely analytical sense. To understand the term in this way, one must of course take into account the specificity of chemical analysis.

1.1 Analytical Context

The analyst aims to receive **the analytical result**, i.e. to identify **the type** (in qualitative analysis) or to determine the **quantity** (in quantitative analysis) of a selected component (**analyte**) in the material (**sample**) assayed. To achieve this goal, he must undertake a series of operations that make up **the analytical procedure**, the general scheme of which is shown in Figure 1.1.

When starting an analysis, the sample must first be prepared for measurement in such a way that its physical and chemical properties are most suitable for measuring the type or amount of analyte in question. This step consists of such processes as, e.g. taking the sample from its natural environment and then changing its aggregate state, diluting it, pre-concentrating it, separating the components, changing the temperature, or causing a chemical reaction.

Calibration in Analytical Science: Methods and Procedures, First Edition. Paweł Kościelniak.

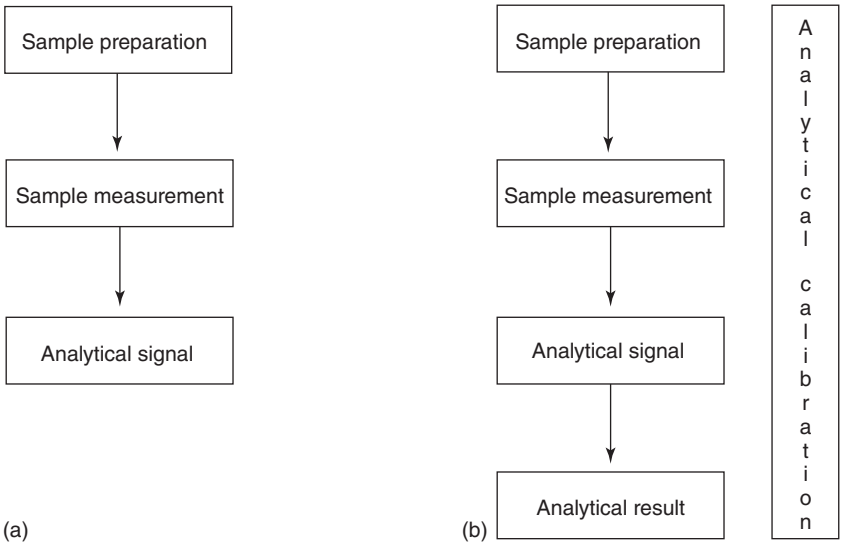

Figure 1.1 Analytical procedure alone (a) and supplemented by analytical calibration (b).

The measurement is generally performed by the chosen using an instrument that operates on the principle of a selected **measurement method** (e.g. atomic absorption spectrometry, potentiometry, etc.). The instrument should respond to the presence of the analyte studied in the form of **measurement signals**. From a calibration point of view, the most relevant signal is the so-called **analytical signal**, i.e. the signal corresponding to the presence of analyte in the sample.

An analytical procedure carried out in a defined manner by a specific measurement method forms an **analytical method**.

The basic analytical problem is that **the analytical signal is not a direct measure of the type and amount of analyte in the sample**, but only information indicating that a certain component in a certain amount is present in the sample. To perform a complete analysis, it is necessary to be able **to transform the analytical signal into the analytical result** and to perform this transformation. This is the role of analytical calibration. As seen in Figure 1.3, the analytical calibration process is an integral part of the analytical procedure and without analytical calibration, qualitative and quantitative analysis cannot be performed. **Realizing this aspect allows one to look at the subject of calibration as a fundamental analytical issue.**

1.2 Principles of Analytical Calibration

However, there is still the question of what the process of transforming an analytical signal to an analytical result consists of, i.e. how analytical calibration should be defined. In this regard, there is also no unified approach, so it is best to rely on official recommendations.

The process of analytical calibration is largely concerned with the making of measurements and the interpretation of measurement data and therefore falls within the scope of metrology. In the Joint Committee for Guides in Metrology (JCGM) document on basic and general terms in metrology, calibration is defined as "… 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" [4]. At the same, the document makes it clear that "calibration should not be confused with adjustment of a measuring system …".

The metrological term, although it allows for a deeper understanding of the concept of calibration, is still rather general because it is inherently applicable to different measurement systems and different types of results obtained. The concept of calibration in the analytical sense is more closely approximated by publications issued by the International Union of Pure and Applied Chemistry (IUPAC). In the paper [5], the IUPAC definition is aligned with the JCGM definition in that it defines analytical calibration as "... the set of operations which establish, under specified conditions, the relationship between value indicated by the analytical instrument and the corresponding known values of an analyte," and in a subsequent IUPAC publication [6] we find an express reference of analytical calibration to both quantitative and qualitative calibration: **"Calibration in analytical chemistry is the operation that determines the functional relationship between measured values (signal intensities at certain signal positions) and analytical quantities characterizing types of analytes and their amount (content, concentration)."**

Such a purely theoretical approach is too general, even abstract, and unrelated to analytical practice. In particular, it does not provide guidance on how the functional relationship (calibration model) should be formulated in different analytical situations and how it relates to the different types of methods used in qualitative and quantitative analysis. Nor does it say anything about the relative nature of the calibration process that the term "measurement standard" gives to the concept in metrological terms.

To extend the definition of analytical calibration, the author proposes to introduce the concept of three functions that relate the signal to the analytical result: the true function, the real function, and the model function [7]. This approach is illustrated in Figure 1.2.

If a sample that an analyst takes for qualitative or quantitative analysis contains a component (analyte) of interest, then before any action is taken with the sample, the type of analyte and its quantity in the sample can be referred to as the **true value** (type or quantity), x_{true}, of the analyte. If it were possible to measure the analytical signal for that analyte at that moment, then the relationship between the resulting signal and its true type or quantity, $Y_{\text{true}} = T(x_{\text{true}})$ could be called the **true function**.

However, the determination of the true function and the true value of the analyte is not possible in practice because it requires the analyst's intervention in the

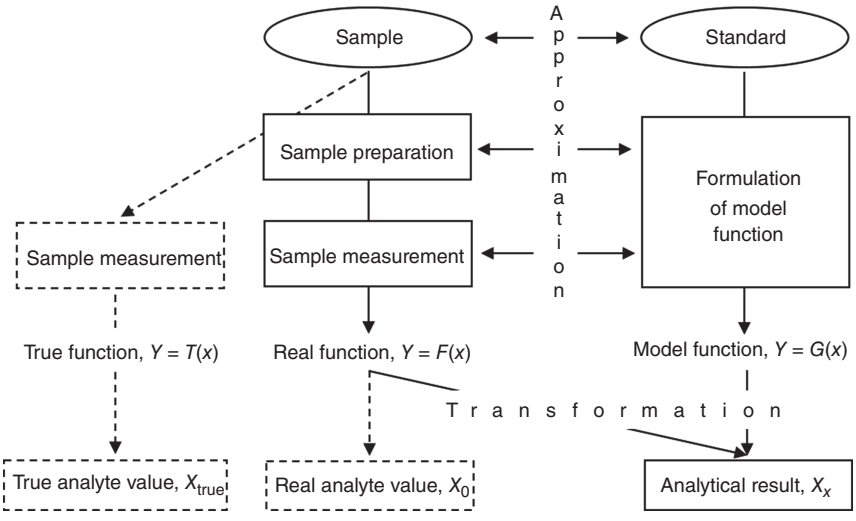

Figure 1.2 Concept of analytical calibration based on the terms of true, $Y = T(x)$, real, $Y = F(x)$, and model, $Y = G(x)$, functions (virtual analytical steps and terms are denoted by dotted lines; for details see text).

form of preparing the sample for measurement and performing the measurement. The initiation of even the simplest and shortest analytical steps results in a change of the true analyte concentration in the sample that continues until the analytical signal is measured. Thus, the concepts of true function and true analyte value are essentially unrealistic and impossible to verify experimentally or mathematically.

When the sample is prepared for analysis, the type or amount of analyte in the sample to be analyzed takes on a **real value, x_0**. The relationship between the analytical signal and the type or amount of analyte is described at this point by the **real function**, $Y = F(x)$, which takes the value Y_0 for the value x_0:

$$Y_0 = F(x_0) \tag{1.1}$$

Although the value of Y_0 is measurable, the exact form of the real function is unknown because it depends on a number of effects and processes that led to the current state of this relationship during the preparation of the sample for measurement. Consequently, the determination of the real result x_0 by means of the real function is impossible.

This situation forces the formulation of an additional auxiliary **model function**, $Y = G(x)$. The role of this function is to replace the real function in the search for the true value, x_0. It should therefore meet two basic conditions: to be known and well-defined and to be the most accurate approximation of the real function ($G(x) \leftrightarrow F(x)$). To fulfill these conditions, a **calibration standard** (one or more) should be used, which should be similar to the sample and properly prepared for measurement.

Assuming that the approximation of the real function by the model function, $Y = G(x)$, is accurate, then the inverse form of the model function, $x = G^{-1}(Y)$, has

to be created, which is called the evaluation function [6]. Theoretically, it allows the value of Y_0 to be transformed into the real result, x_0:

$$x_0 = G^{-1}(Y_0) \tag{1.2}$$

In practice, the approximation of the real function by the model function is never accurate because the real function is essentially unknown. Therefore, transformation (1.2) leads to a certain value x_x:

$$x_x = G^{-1}(Y_0) \tag{1.3}$$

which is an approximate measure of the real result, x_0. This result can also be considered as the final **analytical result**.

The processes of creating a model function and its approximation and transformation are fundamental, integral, and necessary elements of analytical calibration. Thus, it can be said that **analytical calibration consists of approximating the real relationship between the signal, *Y*, and the type, *b*, or amount, *c*, of an analyte in a sample by means of a model function, and then applying this function to transform the signal obtained for the analyte in the sample to the analytical result.**

Note the natural logic of the above description of analytical calibration. Such quantities as "sample" (considered as a collection of unknown chemical constituents), "real function" and "real type or amount of analyte" have their counterparts in the terms of "standard", "model function" and "obtained type or amount of analyte", which are associated with analytical calibration. The former are largely hypothetical, unknown in fact to the analyst, while the latter are known and are approximations of the former. Just as the composition and properties of a sample can never be faithfully reproduced in a standard, the form of the real function cannot be accurately approximated by a model function, and the real type or amount of analyte in the sample at the time the analytical signal is measured can only be approximated by the analytical result obtained.

1.3 Calibration Standards and Models

Depending on the type of univariate model function used, analytical calibration can be broadly divided into **empirical calibration** and **theoretical calibration** [7]. In some cases, the calibration is also of a complex nature to varying degrees (empirical–theoretical or theoretical–empirical) when, to better represent the real function, empirical information is supported by theoretical information or vice versa.

An essential part of any calibration process is the use of calibration standards, which can be of different nature: **chemical, biological, physical, or mathematical** [7]. A common feature of calibration standards is that they directly or indirectly enable the assignment of a measurement signal to a known, well-defined type or amount of analyte. These standards are therefore used to formulate a model function. According to the principle of analytical calibration, a standard should be able to formulate a model function that approximates the true function as closely as possible.

In empirical calibration, the model function is formulated on the basis of the performed experiment, sensory perception, or observation. The sources of information needed to create this type of **empirical model function**, $Y = G(x)$, are measurements of analytical signals obtained directly or indirectly for chemical, biological, or physical standards. In this case, the analyst does not go into the theoretical aspects of the dependence of the analytical signal on the type or amount of analyte (although in some cases the laws and rules underlying this dependence, e.g. Nernst's or Lambert Beer's law, may be helpful).

A widely recognized and used method of analytical calibration is the empirical calibration performed with a **chemical standard**. This is a synthetic or (less commonly) natural material, single or multicomponent, containing an analyte of known type or amount. In special cases, a chemical standard contains a known type or amount of a substance that reacts with the analyte or a known type or amount of an isotope of the element being determined. Calibration with chemical standards is a universal procedure in the sense that it does not depend on the chosen measurement method. The model function formulated is mathematically usually simple and its graphical form is called a calibration graph.

In theoretical calibration, the model function is formulated on the basis of a mathematical description of physicochemical phenomena and processes occurring during the analysis using a given analytical and measurement method. Such a description includes phenomenological quantities based on physical or chemical measurements (electrochemical potentials, diffusion coefficients, etc.), universal quantities (molar mass, atomic number, stoichiometric factors), and/or fundamental physical constants (Faraday constant, Avogadro constant, etc.). The individual elements of the mathematical description act as **mathematical standards**, and the function created with them, $Y = G(x)$, is a **theoretical model function**.

In analytical chemistry, there are relatively few cases of well-defined theoretical models of relatively simple mathematical form. However, in the literature, one can find many new proposals of such functions formulated for various measurement methods. As a rule, they have a very complex mathematical structure, which results from the desire to approximate the real function as accurately as possible. A strong motivation for these scientific efforts is that the theoretical model allows the calculation of the analytical result without the need to prepare chemical standards and perform measurements for the analyte in these standards.

As mentioned, other types of calibration standards can be found in chemical analysis, as well as model functions of a different nature formulated with them, as discussed in Chapter 2 of this chapter. It can be hypothesized that **analytical calibration is inherently connected with the use of standards and the creation of model functions with their help**.

The implications of this approach to analytical calibration are interesting. Qualitative or quantitative analysis performed on the basis of a theoretical model function is often referred to in the literature as calibration-free analysis or absolute analysis. From the point of view of the accepted definition of analytical calibration, this term is misleading, because the formulation of the theoretical model function, like the empirical model, is part of the full calibration procedure. Thus, the questions arise:

can chemical analysis be performed in practice without analytical calibration and what conditions must an analytical method meet to be called a "absolute method"? The discussion of this issue will be the subject of Chapter 2 of this book.

1.4 Calibration Procedures and Methods

The concept of analytical calibration presented above perhaps do not yet give a clear picture of this process. How, then, does the full empirical and theoretical calibration procedure look in general?

As already stated, the calibration process is essential to the performance of chemical analysis – both qualitative and quantitative – and is an integral, inseparable part of any analytical method. What the calibration process contributes to the analytical procedure is the handling of the calibration standard necessary to formulate the model function and use it to transform the measurement signal to the analytical result. Thus, the calibration procedure consists of three steps: preparative, measurement, and transformation.

The **preparative step** consists in preparing the sample and the standard in such a suitable way that the true function, $Y = F(x)$, and the model function, $Y = G(x)$, are similar to each other as much as possible. In the case of empirical z-calibration, there are two main routes to this goal:

- **the sample and standard are prepared separately**, taking care that the chemical composition of the standard is similar to that of the sample and that the preparation of the sample and standard for measurement is similar,
- **the standard is added to the sample** prior to measurement (less frequently prior to sample processing).

In the case of theoretical calibration, separate treatment of the sample and the standard is obvious and natural. Appropriate preparation of the standard in relation to the sample consists in introducing such mathematical standards to the theoretical model that most adequately describe the state of the sample and the phenomena and processes that the sample undergoes under the conditions of the specific measurement method.

In the measurement stage, signal measurements are made using a selected measurement method. If the calibration is empirical, measurements are related to the sample and standard or on the sample and sample with the addition of the standard (depending on their preparation at the preparative stage). In either case, the measurements involving the standard are used to formulate an empirical model function. In the case of a theoretical calibration, measurements are made only for the sample and the formulated theoretical model is considered as the model function.

In the **transformation step**, the value of the signal obtained for the sample is entered into an empirical or theoretical model function and thus the final analytical result (type or amount of analyte in the sample) is determined.

Referring to the formulated extended definition of analytical calibration, it can be noticed that the preparative and measurement stages are used to approximate the

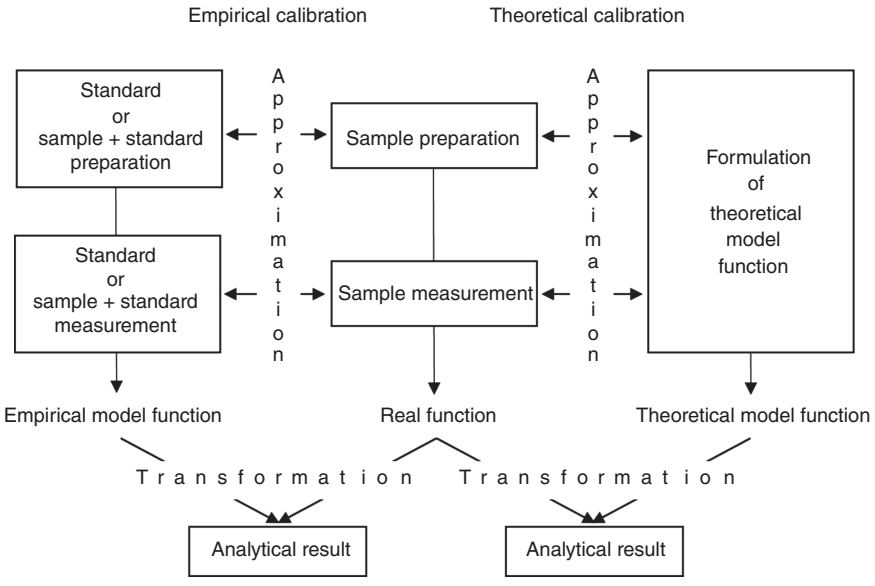

Figure 1.3 General scheme of empirical and theoretical calibration.

model function to the real function, and the key, transformational calibration process takes place at the last stage. A schematic diagram of the procedures of empirical and theoretical calibration is shown in Figure 1.3.

Calibration procedures with specific preparation of sample and standard for measurement form **calibration methods**. In general, therefore, two groups of methods can be distinguished in analytical calibration, which can be called **comparative methods** (when the sample and standard are treated separately) and **additive methods** (when the standard is added to the sample). Within each of these two groups, it is possible to distinguish methods that differ more specifically on the preparative side (e.g. external standard method, internal standard method, standard addition method, etc.). These names are mostly customary and do not always correspond to the specifics of the individual methods. Therefore, another, more essential criterion for the division of the calibration methods in terms of the mathematical way of transforming the measurement signal into the analytical result will also be proposed.

1.5 Calibration in the Context of Measurement Errors

The role of analytical calibration is not only to make it possible to identify or determine an analyte in a sample, but also to do so with as much **accuracy and precision** as possible. The measure of accuracy is the statistically significant difference between the analytical result obtained, x_x, and the true type or amount of analyte, x_{true}, in the sample before it was subjected to any analytical process. The measure of precision is the random difference in analytical results obtained in

so-called parallel analyses, that is, performed in the same way and under the same experimental conditions. The accuracy and precision of an analytical result are thus determined by any systematic and random changes in the true function before it becomes, at the time of measurement, the true function, and then by the systematic and random difference between the true function and its representation, the model function.

Changes in the analytical signal that occur both during sample preparation for measurement and during measurement, resulting in the transformation of the true function to the model function, can be called analytical effects [7]. They can be controllable and uncontrollable. Controlled analytical effects include, for example, changes caused by a targeted action by the analyst to decrease or increase the concentration of an analyte in a sample by dilution or concentration, respectively. Effects of this type can usually be calculated and corrected at the stage of analytical result calculation.

During qualitative and quantitative analysis, however, there are also such changes in the analytical signal that are partially or completely out of the analyst's control. These **uncontrolled analytical effects** can be both random and systematic. Although the analyst is usually aware of the risk of their occurrence and usually tries to prevent them accordingly, he or she may overlook or even neglect them while performing the analysis. As a result, control over the entire analytical process is lost in a sense. Uncontrolled effects manifest themselves by changing the position and intensity of the analytical signal, i.e. they are important in both qualitative and quantitative analysis.

1.5.1 Uncontrolled Analytical Effects

Uncontrolled effects can be caused by many factors manifesting themselves at different stages of the analytical process. The classification of these effects covering all possible factors is, of course, a matter of convention. The division presented below is the author's proposal [7].

Uncontrolled effects are primarily caused by the analyst himself (the so-called **human factor**) as a result of incorrect or careless behavior at various stages of the analytical process. The magnitude of these changes depends primarily on the analyst's knowledge and skills, that is, on his or her professional abilities and qualifications. Personal factors such as tiredness, nervousness, and hurry play a large role. The minimization of the human factor is also not favored by a routine, "automatic" approach to individual analytical activities, resulting, for example, from performing analyses according to a single, unchanging analytical method over a long period of time.

The basic effects include **preparative effect**. Under this term, we understand signal changes caused by such sample processing that results in uncontrolled change (loss, less often gain) of analyte amount in the sample. The analyte can be partially lost e.g. when changing the aggregate state of the sample (to make it suitable for the given measurement method) or when separating its components. The process of changing the amount (or rarely the type) of sample can also take

place outside the purposive, controlled action of the analyst as a result of e.g. an induced chemical reaction. A preparative effect is also involved when the change in signal results directly from physical changes in the sample or standard (e.g. solution viscosity), or from changes in environmental conditions under which the sample and standard are processed (e.g. temperature, humidity, illumination, etc.).

The **instrumental effect** is caused by the action of various instrumental components used in the analytical process to process the sample prior to measurement. In this case, the source of random changes in the analytical signal is all natural imperfections in the design and operation of these instruments, including the measurement systems that characterize the measurement method. However, as a result of instrument malfunction, signal changes can also be systematic.

The instrumental measurement system is the source of separate specific measurement changes occurring in the detection system. This phenomenon can therefore be called a **detection effect**. These changes are manifested, for example, by the limited ability of the system to respond proportionally to the analyte concentration, which is natural for each detector. Another phenomenon is the so-called measurement trend, which consists of a successive increase or decrease in signal intensity over time. In spectrometric methods, there is sometimes the problem of baseline, which varies more or less randomly between spectra. The detection effect can also be related to natural phenomena underlying the measurement method (a typical example is the phenomenon of self-absorption of radiation emitted in the emission spectrometry method, causing a change in analytical signal intensity out of proportion to the amount of analyte in the sample).

The signal measured for a specific type or amount of analyte can also be affected by other components both naturally present in the sample (native) and introduced during sample preparation for measurement. These components then take on the role of interferents, and the signal change caused by them is the so-called **interference effect**. If the effect comes exclusively from the native components of the sample, then it is called a **matrix effect**, while if the interferents are components added to the sample during sample processing, then the induced changes are called a **blank effect**. The interference effect can originate at the stage of sample preparation for measurement (e.g. due to added reagents), but can also be induced during measurement of the analytical signal as a result of phenomena and processes occurring at this stage.

Finally, a specific effect is the **speciation effect**. It occurs when an analyte contained in a sample unexpectedly for the analyst changes its chemical form and at the same time changes its measurement sensitivity. As with the interference effect, this change can occur before measurement (e.g. as a result of a chemical reaction) or at the time of measurement, when it involves a change in that form that is responsible for causing the analytical signal in the detection system (e.g. a change from atoms to analyte ions in atomic absorption spectrometry).

Uncontrolled effects are revealed by a change in the analytical signal either directly or indirectly by changing the type or amount of analyte, as illustrated in Figure 1.4.

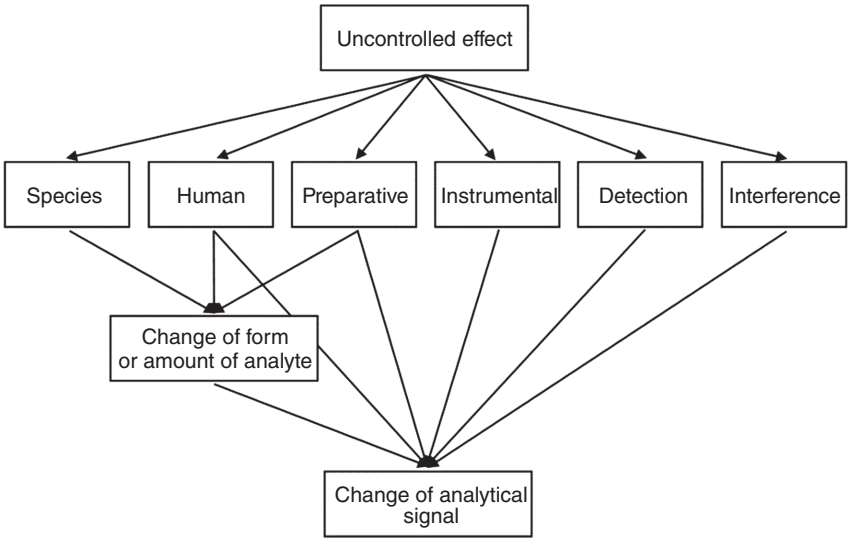

Figure 1.4 Pathways of the various uncontrolled effects. Source: Kościelniak [7]/ Elsevier/CC BY 4.0.

1.5.2 Elimination and Compensation of Uncontrolled Effects

The natural way to avoid uncontrolled effects revealed during sample handling is to employ various means of **eliminating** them. Effectiveness of these actions largely depends on proper identification of the type of these effects and their sources, which are differently situated on the analytical procedure plan. This is shown in Figure 1.5.

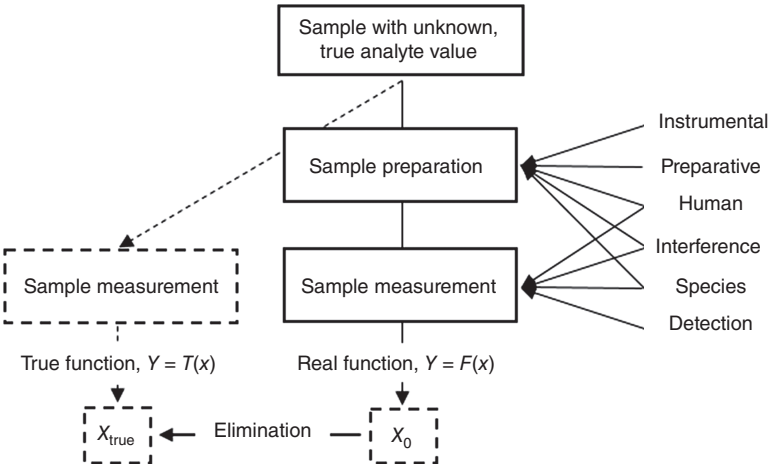

Figure 1.5 Impact of uncontrolled effects on an analyte in the sample during its preparation and measurement; due to elimination of effects the real analyte value approaches the true value ($x_0 \approx x_{true}$). Source: Kościelniak [7]/Elsevier/CC BY 4.0.

The prerequisite for reducing the influence of the human factor is that analyses should be performed only by qualified staff, with a high level of knowledge and skills, maintaining care and caution during the work. Instrumental and detection effects may not be a major problem if the instruments used are of high quality, proven reliability, and low maintenance. In special cases where there are, for example, strong time trends or baseline shifts, special correction procedures are used [8].

In contrast to instrumental and detection effects, speciation effects can be difficult to eliminate if the analytical procedure is relatively complex and involves the use of different types of chemical reactions. The preparative effect can also be difficult to eliminate effectively. This is because no sample processing is in practice free from partial loss of analyte. The degree of this phenomenon should in each case be well recognized by preliminary experiments and then reduced as much as possible. The amount of analyte lost can also be quantified (e.g. by the recovery method, which is discussed later in Chapter 10) and the final analytical result can be corrected on this basis.

The interference effect can be eliminated in basically two ways. The universal way is to remove the interferents from the sample or to isolate the analyte from the sample matrix by appropriately selected laboratory techniques (e.g. by extraction, crystallization, gaseous diffusion, etc.). Another approach is to add an appropriately selected reagent to the sample to eliminate interferents by chemical means.

Progressive elimination of uncontrolled effects causes the two analyte values, true, x_{true}, and real, x_0, to become increasingly similar, as can be seen in Figure 1.5. **When the effects are completely eliminated, the true analyte value becomes an accurate (within random error) measure of the true analyte value in the sample, i.e.** $\boldsymbol{x_0 \approx x_{\text{true}}}$**.**

When proceeding with an analytical calibration, the analyst is forced to use a standard. Importantly, however, this constraint simultaneously provides an opportunity to make the standard similar to the sample. If the sample and standard are similar, then all uncontrolled effects occurring during the analytical procedure should, in theory, manifest themselves in the same way and with appropriate strength with respect to both the sample and the standard. As a result, **compensation** for these effects occurs. Note that effect compensation differs from the process of elimination in that it does not eliminate the uncontrolled effects, but merely involves equalizing them in the sample and standard.

In an empirical calibration performed using a chemical standard, it is easiest to compensate for instrumental effects because it is sufficient to maintain instrumental conditions at the same optimum level during sample and standard preparation for measurement. The detection effect is compensated for just as easily by using the same instrument for both sample and standard measurements and keeping the conditions of the measurements the same.

Compensating for preparative effects is more difficult, although it can be achieved to some extent by subjecting the standard to the same preparative treatments to which the sample was subjected. However, it must be taken into account that the analyte in the standard may be subject to this effect to a different degree than the native analyte due to the different chemical environment and potentially different

chemical form. For speciation effects, it is very important that the chemical form of the analyte remains the same in the sample and in the standard during the calibration procedure. If the analyte is present in several chemical forms in the sample, the analyte in the standard need not take all of these forms but should be in the form in which the analyte is to be determined in the sample.

The most difficult effect to compensate for effectively is the interference effect. It is only relatively simple to compensate for the blank effect by adding the reagents used in sample preparation to the standards. The effect from native sample components requires that the composition of the sample in the standard is accurately reproduced (which is very difficult or even impossible in practice) and that this condition be maintained until measurements are made. However, there are various ways to make the sample and the standard at least partially similar in chemical composition or to compensate for the effect by using an appropriate calibration method. These solutions will be shown and discussed in Chapter 6.

The compensation of effects is offered by the calibration process and is therefore closely related to the representation of the real function by the model function. The more accurate the approximation of the two functions is, the more complete the compensation process is. Progressive compensation of effects promotes a progressive approximation of the analytical result, x_x, to the real result, x_0, as well as the real result, x_0, to the true result, x_{true}. Thus, **after complete compensation, the analytical result becomes an accurate (within random error) estimate of the true value of the analyte in the sample, i.e. $x_x \approx x_{true}$.** This is illustrated schematically in Figure 1.6.

In theoretical calibration, compensating for uncontrolled effects involves describing them adequately by means of a mathematical standard, i.e. including in this description the effects of various factors on the signal measured for the analyte in the sample. However, while a chemical standard can be made similar to a sample due to its similar nature, making a mathematical standard similar to a sample is extremely difficult. Thus, when deciding to use a theoretical calibration, it is

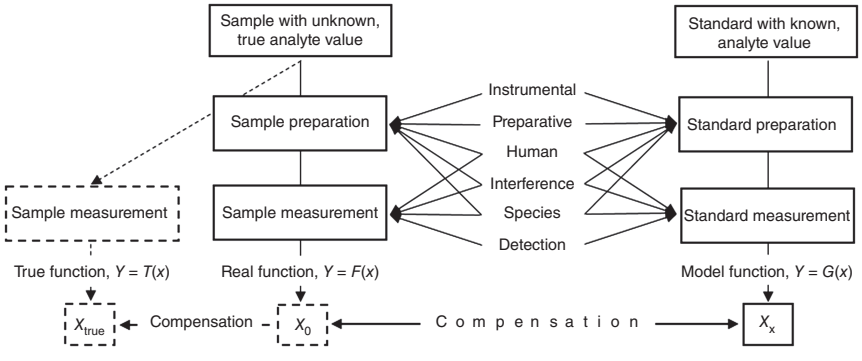

Figure 1.6 Impact of uncontrolled effects on an analyte in both the sample and standard during its preparation and measurement; due to compensation for effects the analytical result value approaches the true value ($x_x \approx x_{true}$). Source: Kościelniak [7]/CC BY 4.0.

important to eliminate, as much as possible, uncontrolled effects affecting the analyte in the sample.

Analytical calibration thus leads to an accurate analytical result either by complete elimination of uncontrolled effects or by their complete compensation. Elimination of an effect thus does not require its compensation (e.g. once an interference effect has been eliminated with a special reagent, there is no need to reconstruct the composition of interferents in the standard), although if it is known that the elimination of an effect may be incomplete, it should be compensated. Similarly, compensation for effects (e.g. instrumental effects) does not require their elimination, although any small reduction increases the chance of their complete compensation. The processes of elimination and compensation of uncontrolled effects are thus complementary activities in the sense that, taken together, they provide the best chance of achieving an accurate assessment of the true value of the analyte in the sample from the analytical result obtained.

So how should the analytical calibration process be evaluated in the context of errors made during the analytical procedure? Certainly, calibration is a potential source of its own random and systematic analytical errors. This is primarily due to the need to use a standard. The empirical standard, like the sample, is subject to uncontrolled effects that may be of a different type than those found in the sample and therefore not compensable. Furthermore, the sample is always more or less different from the standard either because of properties and composition (in empirical calibration) or because of mathematical approximations and corrections (in theoretical calibration). From the imperfection of the calibration standard comes the imperfection of the model function and the added difficulty of accurately approximating the true function.

On the other hand, it should be noted that if it were possible to determine the true value of an analyte in a sample without the contribution of any standard, the analytical procedure used would have to be completely free of uncontrolled effects, or these effects would have to be completely eliminated, and both are impossible in practice. The participation of a calibration standard, i.e. the performance of an analytical calibration, is therefore not only a necessary condition for obtaining an analytical result, but also offers an additional opportunity to improve the quality of this result by compensating for uncontrolled effects.

1.6 Calibration in Qualitative Analysis

Analytical calibration applies equally to qualitative and quantitative analysis [6]. However, in both cases the form of the real function is different, the basis for the formulation of the model function is different, and the accuracy of the results of analyte identification and determination is also evaluated differently. It is therefore worth taking a closer look at these calibration aspects in both types of chemical analysis.

When proceeding with a qualitative analysis, the analyst generally wants to identify the analyte, that is, to find out what component is present in the sample being analyzed or what chemical components the sample is composed of. In some

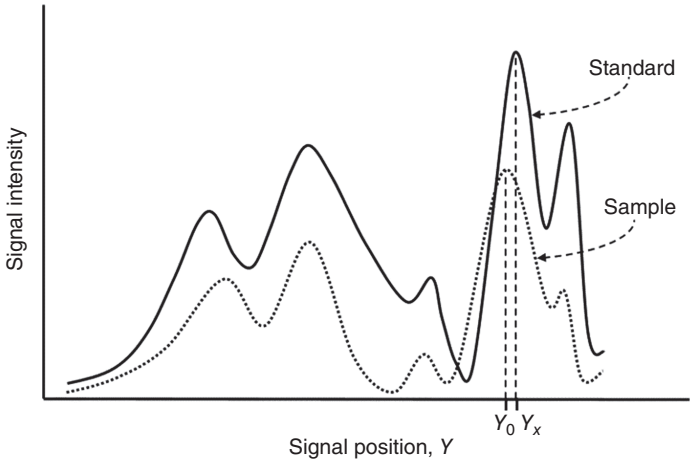

Figure 1.7 Measurement images of the sample and standard used in qualitative analysis: the analyte is identified from the position of the Y_0 and Y_x signals obtained for the unknown analyte in the sample and the known analyte in the standard, respectively.

cases, he asks whether a specific component or several components are present in the sample. In other situations, he may also be interested in questions such as: what is the kind of the whole sample, whether the sample under study is similar to another sample, or whether the sample under study belongs to a particular group of samples.

The relationship between measurement signal and analyte type can be illustrated by the measurement images shown in Figure 1.7. They are created by subjecting a multicomponent sample and a standard of similar chemical composition to the sample to measurements under identical conditions with a specific instrument in such a way that a change in signal intensity is recorded as the specific quantity characteristic of the measurement method used (e.g. wavelength, time, etc.) changes. These signals, when significantly larger than the measurement noise, correspond to the presence of unknown components in the sample and at least one known component, b_x, present in the standard (solid line). **The type of component is indirectly indicated by the signal position** on the abscissa axis, that is, the value of the specific quantity corresponding to the maximum intensity of its signal.

Empirical calibration in qualitative analysis usually involves comparing the signal position value Y_0 obtained for the sample with a similar value Y_x obtained for the standard (see Figure 1.4).[1] Since the value of Y_x obtained for the standard corresponds to the known component b_x, it can be said that both values form a model function, $Y = G(b)$, at a point with coordinates $[Y_x, b_x]$. Because of the similarity of the values of Y_x and Y_0, the real function, $Y = F(b)$, can also be considered as well approximated by the model function at this point. In such a situation, the value of Y_0 is assigned a component b_x using the evaluation function: $b_x = G^{-1}(Y_0)$, and it is

1 This calibration approach refers to a specific comparative calibration method, most commonly used in quantitative analysis.

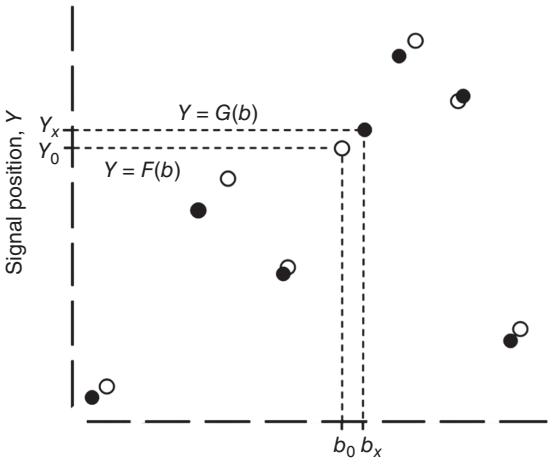

Figure 1.8 Analytical calibration in qualitative analysis: analyte b_0 in a sample (empty points) is identified as analyte b_x in a standard (full points) on the basis of mutual signal positions, Y_0 and Y_x.

claimed that the component b_0 present in the sample is probably the component b_x present in the model. This procedure is illustrated in Figure 1.8.

Theoretical calibration involves the mathematical formulation of a model function, $Y = G(b)$. It should best approximate the real function, $Y = F(b)$, at least in one point with coordinates $[Y_x, b_x]$. When the signal obtained for the sample, Y_0, is substituted into the formula of the inverse function of the formulated model function, the signal position value, b_x, is obtained, indicating the true type, b_0, of the analyte sought.

Mathematically, the real function and the model function are discrete functions in qualitative analysis, as shown in Figure 1.5. When the real function is mapped sufficiently accurately with the model function, any signal of a particular position obtained for known components of the standard is theoretical evidence for the presence or absence of those components in the sample. Some components may be identified by two or more signals,[2] which are analytical signals for them. In many cases, a model function can be used to identify multiple components of a sample, that is, to perform a multicomponent analysis.

When applying the chosen analytical method and recording the signal under appropriately established optimum conditions, the analyst should have at his disposal at least one signal corresponding to a specific type of analyte. It is most advantageous if he has a measurement image of the type shown in Figure 1.7, which covers a wide range of magnitude characterizing the type of constituent. Such an image, obtained under specific experimental conditions, reflects the chemical composition of the entire sample and is characteristic of it. The presence of the desired analyte in the sample can then be indicated not only by the corresponding positions of the analytical signals, but additionally by other parameters, such as the number of these signals, their absolute and relative heights, and even the shape of the entire signal recorded in a given measurement range (some measurement

2 Nevertheless, the relationships $Y = F(b)$ and $Y = G(b)$ can be called functions because, due to the natural random errors of the obtained measured and calculated values, different signals cannot represent perfectly the same measure of a particular sample component.

methods also offer their own specific identification parameters). All these parameters can act as auxiliary identification parameters supporting the basic parameter in the calibration process. A common feature of qualitative analysis is therefore its multi-parametric nature.

Since sample and standard identification parameters are naturally correlated with each other and are highly characteristic of a particular analytical method, the effectiveness of using auxiliary parameters to increase the accuracy of the analytical result with them is limited. Therefore, if there is a need to be more certain about the presence or absence of a sample component (similarity or dissimilarity of samples), then a qualitative analysis of a given sample can be performed by another analytical method (the so-called reference method), preferably as different as possible from the previous one due to the sample processing and measurement method used. In this way, a new range of identification parameter values can be obtained that are not correlated with the previous ones.

A specific aspect of qualitative analysis is the very concept of accuracy of the analytical result. It is clear that if the analyte sought is in the sample or the sample tested is the sample sought (+), and the analytical result confirms this (+), then it is consistent with the actual result, i.e. it is "accurate" ($b_x = b_0$). Similarly, if in such a situation the result obtained is negative (−), it is in fact a false negative, i.e. inaccurate ($b_x \neq b_0$). However, it is, of course, also possible that the analyte sought is not present in the sample or the sample tested is the one sought (+). Then a positive result (+) means that it is in fact inaccurate (false positive, $b_x \neq b_0$), and a negative result (−) means that it is accurate ($b_x = b_0$), although negative. To clearly illustrate these eventualities, they are shown in Table 1.1.

As shown in Figure 1.5, in some cases the degree of similarity of the model function to the real function may raise doubts as to the presence (or absence) of a specific component in the sample, and even this presence (or absence) may possibly rule out. This uncertainty is obviously due to the occurrence of random and systematic uncontrolled effects. Consequently, the accuracy and inaccuracy of an analytical result in a qualitative analysis are always determined with a certain probability, and never with certainty, however certain this certainty may seem to be. By the same token, it cannot be said that a sample and a standard or the two samples

Table 1.1 Estimation of the accuracy and inaccuracy of an analytical result obtained in qualitative analysis.

Analytical result		Evaluation of the obtained result	Accuracy of the result
Obtained, b_x	Real, b_0		
+	+	Truly positive	Accurate
+	−	False positive	Inaccurate
−	+	False negative	Inaccurate
−	−	Truly negative	Accurate

being compared are "the same" but at most "the same," and that they are "certainly different" but "possibly different" with a certain probability.

In qualitative analysis, uncontrolled effects usually manifest themselves as shifts in the position of the analytical signal due to random or systematic changes in instrumental parameters. A frequently occurring problem is also the additive interference effect, consisting of overlapping of signals coming from the analyte and the interferent. The analyte signal may also, under the influence of various factors, so reduce its intensity so that the presence of the analyte may go unnoticed. All these effects will be shown by experimental examples in Chapter 3.

The analytical result in qualitative analysis is nonmeasurable (qualitative) and therefore the assessment of its accuracy is more subjective than in quantitative analysis. This assessment comes down to determining whether and to what extent the differences in measurement information provided by the sample and the standard are statistically significant, i.e. are caused by systematic factors, or are insignificant in comparison with the differences resulting from random errors. However, the multiparameter nature of qualitative analysis means that the use of simple statistical tools (also applicable in quantitative analysis) may be unreliable.

Various chemometric methods that are commercially available in the form of computational packages come to the rescue. These methods are used both to match the model function to the real function as accurately as possible and to assess the accuracy of the result of the identification analysis, which is, therefore, more objective than the analyst's intuitive assessment. However, it should be remembered that it is only up to the analyst to choose the chemometric method and its detailed parameters and criteria on the basis of which it works, and all these factors affect the final results of the calculations. It is not uncommon for two chemometric methods to interpret the same data to produce significantly different results. In such cases, the choice between the results obtained must again be subjective.

Thus, statistical and chemometric approaches to assessing the accuracy of results in qualitative analysis should always be regarded as only auxiliary tools, supporting the knowledge, experience, and research intuition of the analyst.

1.7 Calibration in Quantitative Analysis

The purpose of quantitative analysis is **to establish the amount (content, concentration) of an analyte** (one or more) in the sample being analyzed, that is, the determination of the analyte. Quantitative analysis is therefore formally related to qualitative analysis in the sense that knowledge of the amount of an analyte in a sample is information that naturally supplements the analyst's knowledge of that constituent. On the other hand, the determination of an analyte in a sample in an amount greater than the limit of quantification is at the same time evidence of its presence in that sample. Most often, however, quantitative analysis is undertaken without prior identification of the analyte, predetermining the type of analyte under study and usually knowing the location of the corresponding analytical signal.

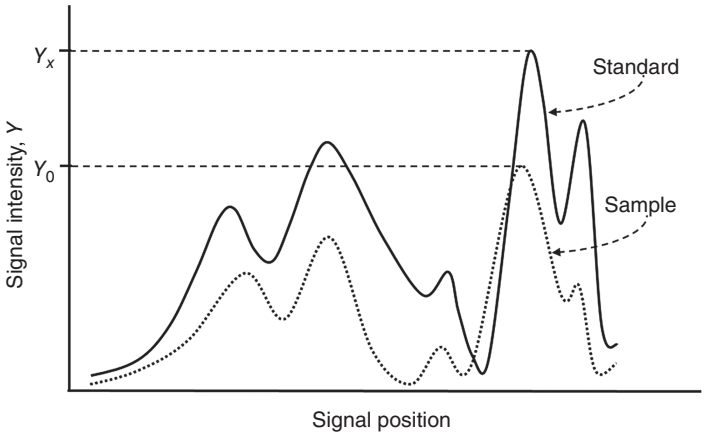

Figure 1.9 Measurement images of the sample and standard used in quantitative analysis: the analyte is determined from the intensities of the Y_0 and Y_x signals obtained for the unknown amount of analyte in the sample and the known amount of analyte in the standard, respectively.

In quantitative analysis, the primary measure of analyte quantity is the intensity[3] of the analytical signal, as can be seen in Figure 1.9. When proceeding to the determination of a specific analyte in a sample, among the possible signals generated by the analyte with a given measuring instrument, the signal with the position at which it shows the highest intensity is selected. If the calibration is empirical, the intensity of the signal measured for a standard (one or more) with a known amount of the analyte is measured under the same conditions (possibly changed only within random error).[4]

In quantitative analysis, the real function is a continuous function because the signal intensity is a continuous quantity. Over a range of analyte amounts, it can take a linear or nonlinear form. Furthermore, in some calibration methods, it is transformed to a decreasing or increasing and decreasing function in different analyte concentration ranges. In such a situation, one cannot count on the values of the intensities of the signals measured for the sample and the single standard being equal (just as the values of the positions of the sample and standard signals are equal in qualitative analysis), i.e. the model function developed using the single standard accurately approximates the true function. If the signal intensities of the sample and standard are significantly different (as in Figure 1.9), the determination of the analyte, although theoretically possible, is risky from the point of view of the accuracy of the analytical result obtained.

Empirical calibration in quantitative analysis, therefore, consists of constructing a model function, $F = G(c)$ in mathematical form from measurements usually made for two or more chemical standards containing the analyte in quantities bounding

3 In some analyses, particularly those detected by separation methods, the area after the signal (peak) is alternatively taken as a measure of analyte quantity.

4 As before, this refers to a specific comparative calibration method, most commonly used in quantitative analysis.

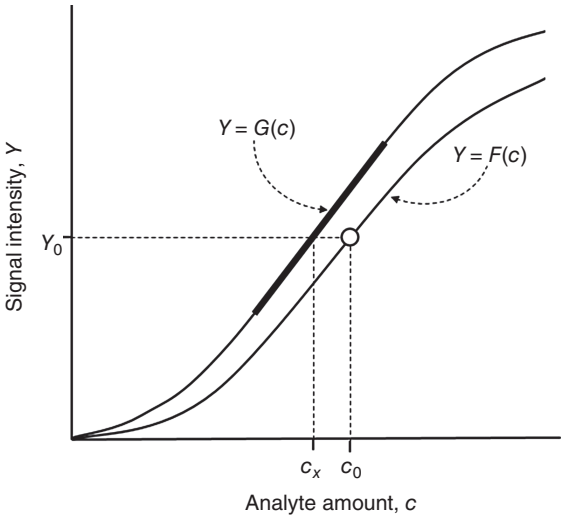

Figure 1.10 Analytical calibration in qualitative analysis: analyte in amount c_0 in the sample (empty point) is determined in amount c_x based on the signal intensity, Y_0, measured for the sample and on the empirical or theoretical model function (bolded line) formulated using chemical or mathematical standards, respectively.

the required range. Usually, a range is chosen in which the model function is most likely to be an exact fit to the linear part of the real function. The amount of analyte in the sample, c_x, is determined from the signal intensity value Y_0 measured for the sample and using the evaluation function: $c_x = G^{-1}(Y_0)$. This procedure is shown in Figure 1.7. As can be seen, the analytical result, c_x, is as close to the true result, c_0, as the model function is to the true function at the point defined by the signal Y_0.

In theoretical calibration, the model $Y = G(c)$ shown in Figure 1.10 is formulated using one or more mathematical formulas. The transformation of the measured signal for the sample, Y_0, to the analytical result, c_x, follows in analogous way as in the empirical calibration.

In quantitative analysis, analytical uncontrolled effects are even more of a problem than in qualitative analysis because the analytical signal is more prone to change its intensity than its position under the influence of various factors. Therefore, the occurrence of any type of effect must be expected during analyte determination. The effect that is particularly problematic in quantitative analysis, but of little significance in qualitative analysis, is the so-called multiplicative interference effect, manifesting itself as a linear or nonlinear change in the intensity of the analytical signal, the greater the concentration of interferents in the sample. Ways of eliminating and compensating for this effect will often be discussed in later chapters.

A separate problem is that uncontrolled effects, regardless of the factors that cause them, are usually manifested by a decrease rather than an increase in the intensity of the analytical signal. Sample processing involves much more loss than gain of analyte (unless the analyst deliberately increases the concentration of analyte in the sample, but then this is a controlled action). The detection effect is generally manifested by a gradual reduction in signal intensity as the analyte concentration in the sample increases. Interferents causing a multiplicative interference effect also tend to cause a gradual reduction in signal intensity. As a consequence, the measurement sensitivity of the analyte is reduced, which is associated with the possibility of larger random errors in quantitative analysis.

Calibration in quantitative analysis is also much more difficult than calibration in qualitative analysis because it requires the formulation of a model function that approximates a continuous real function of unknown shape and position. In addition, it should approximate it not in single points, but in a certain range of analyte amounts, since the amount of analyte in the sample is unknown or can be known only to some approximation. In this situation, the question is justified: how can the **accuracy** of an analytical result obtained by a given analytical method be evaluated in quantitative analysis and how reliable is this evaluation? There are two ways to recommend and use in analytical practice: the application of a reference method or the use of reference material (preferably certified).

The analytical **reference method** should be a well-developed and verified (validated) method to document its high analytical quality. In particular, it should be characterized by a high accuracy of the determination of a given analyte in a given sample. As in qualitative analysis, the method should also be based on different physicochemical principles than the method undergoing accuracy testing. In such a situation, comparison of the analytical result obtained by the reference method with the result obtained for the same analyte in the same sample under analogous experimental conditions by the test method may be a good way to assess the accuracy of the latter. The problem may, of course, be to find a reference method of adequate quality and suitably adapted to the test method.

Another possibility to assess the accuracy of an analytical result is to use a certified reference material [9]. In chemical analysis, there is a substance, usually multicomponent, sufficiently homogeneous and stable, whose chemical composition is determined (at least in part) by interlaboratory analyses and is confirmed by a certificate. Accuracy is tested by selecting a reference material so that it is as similar as possible to the samples analyzed by the method in terms of properties and chemical composition. A sample of the reference material is then analyzed by the method under specified experimental conditions and the result obtained is compared with the certified amount of that analyte. This difference may indicate the accuracy of the method being tested. The problem, of course, is the availability of certified material sufficiently similar to the sample assayed.

In recent years there has been a tendency to use simple chemical standards added to a sample to determine the so-called **analyte recovery** to evaluate the accuracy of analytical results. This approach, although much simpler and less demanding than the methods described above, is nevertheless fallible and can only be used under strictly defined conditions. This will be demonstrated in a later chapter of this book devoted entirely to this subject.

In quantitative analysis, particularly important parameters that testify to the quality of an analytical method (so-called validation parameters) are, in addition to accuracy, precision, and uncertainty of the analytical result.

Precision is assessed by the random scatter of the analytical result, i.e. the values of that result determined several times by a given analytical method under the same or only slightly changed experimental conditions. This is expressed in the form of **repeatability** and **reproducibility.** The former is the precision established under conditions in which the analytical procedure is performed according to the

specified analytical method by the same analyst, using the same equipment, and in the shortest possible time (at most one day). The latter is the precision established under conditions in which one of the above factors (analyst, equipment, day) has been deliberately changed.

It should be noted that a precision determined – as is quite often done – solely on the basis of the scatter of only the measurement results determined with the sample cannot be regarded as a measure of the precision of the analytical result, much less as a measure of the quality of the analytical method. Such a way of proceeding ignores the contribution that the calibration procedure, that is, the preparation and measurement of standards and the transformation of the measurement signal to the analytical result, makes to the general precision value.

Uncertainty is defined as the interval within which the value of an analytical result can be located with satisfactory probability [10]. The overall value of uncertainty consists of the component uncertainties with which the various steps and actions that make up the analytical procedure are performed. Some of these component values can be calculated as experimental standard deviations from the statistical distribution of the results of a series of measurements. Other values, which may also be characterized by standard deviations, are evaluated from assumed probability distributions based on the analyst's experience or the information available to the analyst. It is obvious that all steps of the calibration procedure should be included in the evaluation of the overall uncertainty of the analytical result.

1.8 General Rules for Correct Calibration

Based on the above considerations, one may be tempted to define some general rules of conduct that will allow the calibration process to be carried out primarily so that the analytical result is subject to the lowest possible random and systematic errors. These rules should also be as consistent as possible with the rules of green analytical chemistry [11]. This means that when determining the correct calibration procedure, the analyst should take into account the minimization of factors that pose a threat to our environment.

The first very important factor is the **selection of an appropriate analytical method**. If the analyst has several qualitatively equal methods at his disposal leading to the identification or determination of an analyte in a given sample or is starting to develop a new analytical method, he should take care that the chosen or developed method is **as simple as possible** chemically and instrumentally. Several factors support this. A simple analytical method contains relatively few sources of uncontrolled effects, which promotes their effective elimination. It is also important that the simpler the method, the easier and more accurately the standard's handling can be made to resemble that of the sample, and, as a result, uncontrolled effects can be effectively compensated for. The simpler the analytical method, the greater the chance of using fewer and less reagents and producing little waste, i.e. of following the basic rules of green analytical chemistry.

Irrespective of the analytical method chosen, it is essential that all the steps required by the analytical method are carried out with the **greatest possible**

correctness and care – i.e. in accordance with all the rules of the analytical art. The human factor plays an important role in both empirical calibration and theoretical calibration. The sources of random and systematic errors associated with the construction of the empirical model function are all incorrectly or carelessly performed laboratory operations, and in theoretical calibration – incorrect theoretical assumptions, erroneous or inaccurate calculations, and all approximations with which the mathematical description of phenomena and processes is made. Note that personal errors can dominate the error of an analytical result regardless of other steps taken to eliminate or compensate for uncontrolled effects.

During the implementation of an analytical method, a very important issue is the skillful, balanced use of both ways: **elimination and compensation of uncontrolled effects**. In particular, this applies to empirical calibration with chemical standards. This is because it is supported by theoretical and practical considerations outlined above. Both ways should be used in such a way that they complement each other and are as effective as possible. It is best to be guided by specific, proven, and customary principles as well as by one's own analytical experience.

Thus, for example,

- during the analysis measurements for sample and standard should be made under identical experimental conditions set at optimum levels and with the same measuring instrument,
- do not use too many reagents to eliminate uncontrolled (preparative, interference) effects (especially those endangering health and life), but rather try to compensate for these effects,
- try as far as possible to make the chemical composition of the standards similar to that of the sample (e.g. by means of reference materials) and treat the standards in the same way as the sample,
- pay close attention to the compatibility of the chemical form of the analyte in the sample and in the standard and in case of doubt take appropriate instrumental or chemical steps to ensure this compatibility.

The same guidelines also apply to theoretical calibration, though of course taking into account the appropriate chemical (relative to the sample) and mathematical (relative to the standard) ways.

It is important to remember that the calibration process is inherently linked to the overall analytical process. It is therefore important that all calibration activities are performed in a **correct and careful manner**, just like other non-calibration operations. This seems to be a trivial and obvious statement, but reality often does not bear this out. As shown in scientific publications, analysts directing their efforts to create new and ingenious analytical procedures often neglect the calibration aspects. It is not uncommon, therefore, that the lack of a proposal for a clearly defined, suitably adapted calibration procedure within the analytical procedure developed is, in effect, the cause of unsatisfactory results in terms of their accuracy and precision.

Most random and systematic errors are made at the stage of sample and standard preparation for measurements. Minimization of these errors is facilitated by **mechanization and automation** of this stage of the analytical procedure. One way of

doing this in quantitative analysis is to process the sample and standard in flow mode, examples of which will be presented in later chapters of this book. The automation of analysis not only allows, as will be shown, for an improvement in the quality parameters of the analytical method but also provides greater operational safety and supports lower reagent consumption and waste reduction. It is therefore very important to implement calibration procedures into analytical methods performed in such a mode.

Finally, one more matter of importance in the context of the subject of this book should be mentioned: **the proper choice of the calibration method**. This is, in fact, one of the most important factors determining the correctness of the whole analytical procedure, since it affects not only the precision and uncertainty of the analytical result but also its accuracy. However, in order for this choice to be appropriate, adequate to the different circumstances in which the analysis is carried out, it is necessary to have a good knowledge of the different calibration methods, sometimes very rare, but applicable in qualitative and quantitative analysis. The acquisition of this knowledge is precisely the main purpose of this book.

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