1.1. THE MEASUREMENT PROCESS

The purpose of an analytical study is to obtain information about some object or substance. The substance could be a solid, a liquid, a gas, or a biological material. The information to be obtained can be varied. It could be the chemical or physical composition, structural or surface properties, or a sequence of proteins in genetic material. Despite the sophisticated arsenal of analytical techniques available, it is not possible to find every bit of information of even a very small number of samples. For the most part, the state of current instrumentation has not evolved to the point where we can take an instrument to an object and get all the necessary information. Although there is much interest in such noninvasive devices, most analysis is still done by taking a part (or portion) of the object under study (referred to as the sample) and analyzing it in the laboratory (or at the site). Some common steps involved in the process are shown in Figure 1.1.

The first step is **sampling**, where the sample is obtained from the object to be analyzed. This is collected such that it represents the original object. Sampling is done with variability within the object in mind. For example, while collecting samples for determination of Ca\(^{2+}\) in a lake, it should be kept in mind that its concentrations can vary depending on the location, the depth, and the time of year.

The next step is **sample preservation**. This is an important step, because there is usually a delay between sample collection and analysis. Sample preservation ensures that the sample retains its physical and chemical characteristics so that the analysis truly represents the object under study. Once
the sample is ready for analysis, *sample preparation* is the next step. Most samples are not ready for direct introduction into instruments. For example, in the analysis of pesticides in fish liver, it is not possible to analyze the liver directly. The pesticides have to be extracted into a solution, which can be analyzed by an instrument. There might be several processes within sample preparation itself. Some steps commonly encountered are shown in Figure 1.2. However, they depend on the sample, the matrix, and the concentration level at which the analysis needs to be carried out. For instance, trace analysis requires more stringent sample preparation than major component analysis.

Once the sample preparation is complete, the analysis is carried out by an instrument of choice. A variety of instruments are used for different types of analysis, depending on the information to be acquired: for example, chromatography for organic analysis, atomic spectroscopy for metal analysis, capillary electrophoresis for DNA sequencing, and electron microscopy for small structures. Common analytical instrumentation and the sample preparation associated with them are listed in Table 1.1. The sample preparation depends on the analytical techniques to be employed and their capabilities. For instance, only a few microliters can be injected into a gas chromatograph. So in the example of the analysis of pesticides in fish liver, the ultimate product is a solution of a few microliters that can be injected into a gas chromatograph. Sampling, sample preservation, and sample preparation are
all aimed at producing those few microliters that represent what is in the fish. It is obvious that an error in the first three steps cannot be rectified by even the most sophisticated analytical instrument. So the importance of the prior steps, in particular the sample preparation, cannot be understressed.

1.1.1. Qualitative and Quantitative Analysis

There is seldom a unique way to design a measurement process. Even an explicitly defined analysis can be approached in more than one ways. Different studies have different purposes, different financial constraints, and are carried out by staff with different expertise and personal preferences. The most important step in a study design is the determination of the purpose, and at least a notion of the final results. It should yield data that provide useful information to solve the problem at hand.

The objective of an analytical measurement can be qualitative or quantitative. For example, the presence of pesticide in fish is a topic of concern. The questions may be: Are there pesticides in fish? If so, which ones? An analysis designed to address these questions is a qualitative analysis, where the analyst screens for the presence of certain pesticides. The next obvious question is: How much pesticide is there? This type of analysis, quantitative analysis, not only addresses the presence of the pesticide, but also its concentration. The other important category is semiqualitative analysis. Here
the concern is not exactly how much is there but whether it is above or below a certain threshold level. The prostate specific antigen (PSA) test for the screening of prostate cancer is one such example. A PSA value of 4 ng/L (or higher) implies a higher risk of prostate cancer. The goal here is to determine if the PSA is higher or lower than 4 ng/L.

Once the goal of the analyses and target analytes have been identified, the methods available for doing the analysis have to be reviewed with an eye to accuracy, precision, cost, and other relevant constraints. The amount of labor, time required to perform the analysis, and degree of automation can also be important.

### 1.1.2. Methods of Quantitation

Almost all measurement processes, including sample preparation and analysis, require calibration against chemical standards. The relationship between a detector signal and the amount of analyte is obtained by recording

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample Preparation</th>
<th>Instrumenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organics</td>
<td>Extraction, concentration, cleanup, derivatization</td>
<td>GC, HPLC, GC/MS, LC/MS</td>
</tr>
<tr>
<td>Volatile organics</td>
<td>Transfer to vapor phase, concentration</td>
<td>GC, GC-MS</td>
</tr>
<tr>
<td>Metals</td>
<td>Extraction, concentration, speciation</td>
<td>AA, GFAA, ICP, ICP/MS</td>
</tr>
<tr>
<td>Metals</td>
<td>Extraction, derivatization, concentration, speciation</td>
<td>UV-VIS molecular absorption spectrophotometry, ion chromatography</td>
</tr>
<tr>
<td>Ions</td>
<td>Extraction, concentration, derivatization</td>
<td>IC, UV-VIS</td>
</tr>
<tr>
<td>DNA/RNA</td>
<td>Cell lysis, extraction, PCR</td>
<td>Electrophoresis, UV-VIS, fluorescence</td>
</tr>
<tr>
<td>Amino acids, fats, carbohydrates</td>
<td>Extraction, cleanup</td>
<td>GC, HPLC, electrophoresis</td>
</tr>
<tr>
<td>Microstructures</td>
<td>Etching, polishing, reactive ion techniques, ion bombardments, etc.</td>
<td>Microscopy, surface spectrometry</td>
</tr>
</tbody>
</table>

*a GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; AA, atomic absorption; GFAA, graphite furnace atomic absorption; ICP, inductively coupled plasma; UV-VIS, ultraviolet–visible molecular absorption spectrophotometry; IC, ion chromatography.*
the response from known quantities. Similarly, if an extraction step is involved, it is important to add a known amount of analyte to the matrix and measure its recovery. Such processes require standards, which may be prepared in the laboratory or obtained from a commercial source. An important consideration in the choice of standards is the matrix. For some analytical instruments, such as x-ray fluorescence, the matrix is very important, but it may not be as critical for others. Sample preparation is usually matrix dependent. It may be easy to extract a polycyclic aromatic hydrocarbon from sand by supercritical extraction but not so from an aged soil with a high organic content.

**Calibration Curves**

The most common calibration method is to prepare standards of known concentrations, covering the concentration range expected in the sample. The matrix of the standard should be as close to the samples as possible. For instance, if the sample is to be extracted into a certain organic solvent, the standards should be prepared in the same solvent. The calibration curve is a plot of detector response as a function of concentration. A typical calibration curve is shown in Figure 1.3. It is used to determine the amount of analyte in the unknown samples. The calibration can be done in two ways, best illustrated by an example. Let us say that the amount of lead in soil is being measured. The analytical method includes sample preparation by acid extraction followed by analysis using atomic absorption (AA). The stan-

![Figure 1.3. Typical calibration curve.](image-url)
standards can be made by spiking clean soil with known quantities of lead. Then the standards are taken through the entire process of extraction and analysis. Finally, the instrument response is plotted as a function of concentration. The other option assumes quantitative extraction, and the standards are used to calibrate only the AA. The first approach is more accurate; the latter is simpler. A calibration method that takes the matrix effects into account is the method of standard addition, which is discussed briefly in Chapter 4.

1.2. ERRORS IN QUANTITATIVE ANALYSIS: ACCURACY AND PRECISION

All measurements are accompanied by a certain amount of error, and an estimate of its magnitude is necessary to validate results. The error cannot be eliminated completely, although its magnitude and nature can be characterized. It can also be reduced with improved techniques. In general, errors can be classified as random and systematic. If the same experiment is repeated several times, the individual measurements cluster around the mean value. The differences are due to unknown factors that are stochastic in nature and are termed random errors. They have a Gaussian distribution and equal probability of being above or below the mean. On the other hand, systematic errors tend to bias the measurements in one direction. Systematic error is measured as the deviation from the true value.

1.2.1. Accuracy

Accuracy, the deviation from the true value, is a measure of systematic error. It is often estimated as the deviation of the mean from the true value:

$$\text{accuracy} = \frac{\text{mean} - \text{true value}}{\text{true value}}$$

The true value may not be known. For the purpose of comparison, measurement by an established method or by an accredited institution is accepted as the true value.

1.2.2. Precision

Precision is a measure of reproducibility and is affected by random error. Since all measurements contain random error, the result from a single measurement cannot be accepted as the true value. An estimate of this error is necessary to predict within what range the true value may lie, and this is done
by repeating a measurement several times [1]. Two important parameters, the
average value and the variability of the measurement, are obtained from this
process. The most widely used measure of average value is the arithmetic
mean, $\bar{x}$:

$$\bar{x} = \frac{\sum x_i}{n}$$

where $\sum x_i$ is the sum of the replicate measurements and $n$ is the total
number of measurements. Since random errors are normally distributed, the
common measure of variability (or precision) is the standard deviation, $\sigma$.
This is calculated as

$$\sigma = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n}}$$  \hspace{1cm} (1.1)$$

When the data set is limited, the mean is often approximated as the true
value, and the standard deviation may be underestimated. In that case, the
unbiased estimate of $\sigma$, which is designated $s$, is computed as follows:

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$  \hspace{1cm} (1.2)$$

As the number of data points becomes larger, the value of $s$ approaches
that of $\sigma$. When $n$ becomes as large as 20, the equation for $\sigma$ may be
used. Another term commonly used to measure variability is the coefficient
of variation (CV) or relative standard deviation (RSD), which may also be
expressed as a percentage:

$$\text{RSD} = \frac{s}{\bar{x}} \quad \text{or} \quad \% \text{RSD} = \frac{s}{\bar{x}} \times 100$$  \hspace{1cm} (1.3)$$

Relative standard deviation is the parameter of choice for expressing preci-
sion in analytical sciences.

Precision is particularly important when sample preparation is involved.
The variability can also affect accuracy. It is well known that reproduc-
bility of an analysis decreases disproportionately with decreasing concen-
tration [2]. A typical relationship is shown in Figure 1.4, which shows
that the uncertainty in trace analysis increases exponentially compared to
the major and minor component analysis. Additional deviations to this
curve are expected if sample preparation steps are added to the process. It
may be prudent to assume that uncertainty from sample preparation would
also increase with decrease in concentration. Generally speaking, analytical
instruments have become quite sophisticated and provide high levels of accuracy and precision. On the other hand, sample preparation often remains a rigorous process that accounts for the majority of the variability. Going back to the example of the measurement of pesticides in fish, the final analysis may be carried out in a modern computer-controlled gas chromatograph/mass spectrograph (GC-MS). At the same time, the sample preparation may involve homogenization of the liver in a grinder, followed by Soxhlett extraction, concentration, and cleanup. The sample preparation might take days, whereas the GC-MS analysis is complete in a matter of minutes. The sample preparation also involves several discrete steps that involve manual handling. Consequently, both random and systematic errors are higher during sample preparation than during analysis.

The relative contribution of sample preparation depends on the steps in the measurement process. For instance, typically two-thirds of the time in an analytical chromatographic procedure is spent on sample preparation. An example of the determination of olanzapine in serum by high-performance liquid chromatography/mass spectroscopy (HPLC-MS) illustrates this point [3]. Here, samples were mixed with an internal standard and cleaned up in a

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**Figure 1.4.** Reproducibility as a function of concentration during analytical measurements. (Reproduced from Ref. 3 with permission from LC-GC North America.)
solid-phase extraction (SPE) cartridge. The quantitation was done by a calibration curve. The recovery was $87 \pm 4\%$ for three assays, whereas repeatability of 10 replicate measurements was only 1 to 2\%. A detailed error analysis [3] showed that 75\% of the uncertainty came from the SPE step and the rest came from the analytical procedure. Of the latter, 24\% was attributed to uncertainty in the calibration, and the remaining 1\% came from the variation in serum volume. It is also worth noting that improvement in the calibration procedure can be brought about by measures that are significantly simpler than those required for improving the SPE. The variability in SPE can come from the cartridge itself, the washing, the extraction, the drying, or the redissolution steps. There are too many variables to control.

Some useful approaches to reducing uncertainty during sample preparation are given below.

**Minimize the Number of Steps**

In the example above, the sample preparation contributed 75\% of the error. When multiple steps such as those shown in Figure 1.2 are involved, the uncertainty is compounded. A simple dilution example presented in Figure 1.5 illustrates this point. A 1000-fold dilution can be performed in one step: 1 mL to 1000 mL. It can also be performed in three steps of 1:10 dilutions each. In the one-step dilution, the uncertainty is from the uncertainty in the volume of the pipette and the flask. In the three-step dilution, three pipettes and three flasks are involved, so the volumetric uncertainty is compounded that many times. A rigorous analysis showed [3] that the uncertainty in the one-step dilution was half of what was expected in the three-step process.

If and when possible, one or more sample preparation steps (Figure 1.2) should be eliminated. The greater the number of steps, the more errors there are. For example, if a cleanup step can be eliminated by choosing a selective extraction procedure, that should be adapted.

**Use Appropriate Techniques**

Some techniques are known to provide higher variability than others. The choice of an appropriate method at the outset can improve precision. For example, a volume of less than 20 mL can be measured more accurately and precisely with a syringe than with a pipette. Large volumes are amenable to precise handling but result in dilution that lowers sensitivity. The goal should be to choose a combination of sample preparation and analytical instrumentation that reduces both the number of sample preparative steps and the RSD. Automated techniques with less manual handling tend to have higher precision.
Uncertainty in a method can come from both the sample preparation and the analysis. The total variance is the sum of the two factors:

\[ \sigma_T^2 = \sigma_s^2 + \sigma_a^2 \]  

The subscript \( T \) stands for the total variance; the subscripts \( s \) and \( a \) stand for the sample preparation and the analysis, respectively. The variance of the analytical procedure can be subtracted from the total variance to estimate the variance from the sample preparation. This could have contribution from the steps shown in Figure 1.2:

\[ \sigma_s^2 = \sigma_h^2 + \sigma_{ex}^2 + \sigma_c^2 + \sigma_{cl}^2 \]  

where \( \sigma_h \) relates to homogenization, \( \sigma_{ex} \) to extraction, \( \sigma_c \) to concentration, and \( \sigma_{cl} \) to cleanup. Consequently, the overall precision is low even when

Figure 1.5. Examples of single and multiple dilution of a sample. (Reproduced from Ref. 3 with permission from LC-GC North America.)

### 1.2.3. Statistical Aspects of Sample Preparation

Uncertainty in a method can come from both the sample preparation and the analysis. The total variance is the sum of the two factors:

\[ \sigma_T^2 = \sigma_s^2 + \sigma_a^2 \]  

The subscript \( T \) stands for the total variance; the subscripts \( s \) and \( a \) stand for the sample preparation and the analysis, respectively. The variance of the analytical procedure can be subtracted from the total variance to estimate the variance from the sample preparation. This could have contribution from the steps shown in Figure 1.2:

\[ \sigma_s^2 = \sigma_h^2 + \sigma_{ex}^2 + \sigma_c^2 + \sigma_{cl}^2 \]  

where \( \sigma_h \) relates to homogenization, \( \sigma_{ex} \) to extraction, \( \sigma_c \) to concentration, and \( \sigma_{cl} \) to cleanup. Consequently, the overall precision is low even when
a high-precision analytical instrument is used in conjunction with low-precision sample preparation methods. The total variance can be estimated by repeating the steps of sample preparation and analysis several times.

Usually, the goal is to minimize the number of samples, yet meet a specific level of statistical certainty. The total uncertainty, $E$, at a specific confidence level is selected. The value of $E$ and the confidence limits are determined by the measurement quality required:

$$E = \frac{z\sigma}{\sqrt{n}}$$

where $\sigma$ is the standard deviation of the measurement, $z$ the percentile of standard normal distribution, depending on the level of confidence, and $n$ the number of measurements. If the variance due to sample preparation, $\sigma_s^2$, is negligible and most of the uncertainty is attributed to the analysis, the minimum number of analysis per sample is given by

$$n_a = \left(\frac{z\sigma_a}{E_a}\right)^2$$

The number of analyses can be reduced by choosing an alternative method with higher precision (i.e., a lower $\sigma_a$) or by using a lower value of $z$, which means accepting a higher level of error. If the analytical uncertainty is negligible ($\sigma_a \rightarrow 0$) and sample preparation is the major issue, the minimum number of samples, $n_s$, is given by

$$n_s = \left(\frac{z\sigma_s}{E_s}\right)^2$$

Again, the number of samples can be reduced by accepting a higher uncertainty or by reducing $\sigma_s$. When $\sigma_a$ and $\sigma_s$ are both significant, the total error $E_T$ is given by

$$E_T = z\left(\frac{\sigma_a^2}{n_s} + \frac{\sigma_s^2}{n_a}\right)^{1/2}$$

This equation does not have an unique solution. The same value of error, $E_T$, can be obtained by using different combinations of $n_s$ and $n_a$. Combinations of $n_s$ and $n_a$ should be chosen based on scientific judgment and the cost involved in sample preparation and analysis.
A simple approach to estimating the number of samples is to repeat the sample preparation and analysis to calculate an overall standard deviation, \( s \). Using Student’s \( t \) distribution, the number of samples required to achieve a given confidence level is calculated as

\[
\frac{ts}{e}^2
\]

where \( t \) is the \( t \)-statistic value selected for a given confidence level and \( e \) is the acceptable level of error. The degrees of freedom that determine \( t \) can first be chosen arbitrarily and then modified by successive iterations until the number chosen matches the number calculated.

**Example**

Relative standard deviation of repeat HPLC analysis of a drug metabolite standard was between 2 and 5%. Preliminary measurements of several serum samples via solid-phase extraction cleanup followed by HPLC analyses showed that the analyte concentration was between 5 and 15 mg/L and the standard deviation was 2.5 mg/L. The extraction step clearly increased the random error of the overall process. Calculate the number of samples required so that the sample mean would be within \( \pm 1.2 \) mg/L of the population mean at the 95% confidence level.

Using equation (1.10), assuming 10 degrees of freedom, and referring to the \( t \)-distribution table from a statistics textbook, we have \( t = 2.23 \), \( s = 2.5 \), and \( e = 1.2 \) mg/L, so \( n = (2.23 \times 2.5 / 1.2)^2 = 21.58 \) or 22. Since 22 is significantly larger than 10, a correction must be made with the new value of \( t \) corresponding to 21 degrees of freedom (\( t = 2.08 \)): \( n = (2.08 \times 2.5 / 1.2)^2 = 18.78 \) or 19. Since 19 and 22 are relatively close, approximately that many samples should be tested. A higher level of error, or a lower confidence level, may be accepted for the reduction in the number of samples.

### 1.3. METHOD PERFORMANCE AND METHOD VALIDATION

The criteria used for evaluating analytical methods are called *figures of merit*. Based on these characteristics, one can predict whether a method meets the needs of a certain application. The figures of merit are listed in Table 1.2. Accuracy and precision have already been discussed; other important characteristics are sensitivity, detection limits, and the range of quantitation.
1.3.1. Sensitivity

The sensitivity of a method (or an instrument) is a measure of its ability to distinguish between small differences in analyte concentrations at a desired confidence level. The simplest measure of sensitivity is the slope of the calibration curve in the concentration range of interest. This is referred to as the *calibration sensitivity*. Usually, calibration curves for instruments are linear and are given by an equation of the form

\[ S = mc + s_{bl} \]  \hspace{1cm} (1.11)

where \( S \) is the signal at concentration \( c \) and \( s_{bl} \) is the blank (i.e., signal in the absence of analyte). Then \( m \) is the slope of the calibration curve and hence the sensitivity. When sample preparation is involved, recovery of these steps has to be factored in. For example, during an extraction, only a fraction proportional to the extraction efficiency \( r \) is available for analysis. Then equation (1.11) reduces to

\[ S = mrc + s_{bl} \]  \hspace{1cm} (1.12)

Now the sensitivity is \( m r \) rather than \( m \). The higher the recovery, the higher the sensitivity. Near 100% recovery ensures maximum sensitivity. The

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### Table 1.2. Figures of Merit for Instruments or Analytical Methods

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Accuracy</td>
<td>Deviation from true value</td>
</tr>
<tr>
<td>2</td>
<td>Precision</td>
<td>Reproducibility of replicate measurements</td>
</tr>
<tr>
<td>3</td>
<td>Sensitivity</td>
<td>Ability to discriminate between small differences in concentration</td>
</tr>
<tr>
<td>4</td>
<td>Detection limit</td>
<td>Lowest measurable concentration</td>
</tr>
<tr>
<td>5</td>
<td>Linear dynamic range</td>
<td>Linear range of the calibration curve</td>
</tr>
<tr>
<td>6</td>
<td>Selectivity</td>
<td>Ability to distinguish the analyte from interferances</td>
</tr>
<tr>
<td>7</td>
<td>Speed of analysis</td>
<td>Time needed for sample preparation and analysis</td>
</tr>
<tr>
<td>8</td>
<td>Throughput</td>
<td>Number of samples that can be run in a given time period</td>
</tr>
<tr>
<td>9</td>
<td>Ease of automation</td>
<td>How well the system can be automated</td>
</tr>
<tr>
<td>10</td>
<td>Ruggedness</td>
<td>Durability of measurement, ability to handle adverse conditions</td>
</tr>
<tr>
<td>11</td>
<td>Portability</td>
<td>Ability to move instrument around</td>
</tr>
<tr>
<td>12</td>
<td>Greenness</td>
<td>Ecoefficiency in terms of waste generation and energy consumption</td>
</tr>
<tr>
<td>13</td>
<td>Cost</td>
<td>Equipment cost + cost of supplies + labor cost</td>
</tr>
</tbody>
</table>
blank is also modified by the sample preparation step; $s_{\text{bl}}$ refers to the blank that arises from total contribution from sample preparation and analysis.

Since the precision decreases at low concentrations, the ability to distinguish between small concentration differences also decreases. Therefore, sensitivity as a function of precision is measured by *analytical sensitivity*, which is expressed as [4]

$$a = \frac{mr}{s_t} \quad (1.13)$$

where $s_t$ is the standard deviation based on sample preparation and analysis. Due to its dependence on $s_t$, analytical sensitivity varies with concentration.

### 1.3.2. Detection Limit

The *detection limit* is defined as the lowest concentration or weight of analyte that can be measured at a specific confidence level. So, near the detection limit, the signal generated approaches that from a blank. The detection limit is often defined as the concentration where the signal/noise ratio reaches an accepted value (typically, between 2 and 4). Therefore, the smallest distinguishable signal, $S_m$, is

$$S_m = X_{\text{bl}} + ks_{\text{bl}} \quad (1.14)$$

where, $X_{\text{bl}}$ and $s_{\text{bl}}$ are the average blank signal and its standard deviation. The constant $k$ depends on the confidence level, and the accepted value is 3 at a confidence level of 89%. The detection limit can be determined experimentally by running several blank samples to establish the mean and standard deviation of the blank. Substitution of equation (1.12) into (1.14) and rearranging shows that

$$C_m = \frac{S_m - s_{\text{bl}}}{m} \quad (1.15)$$

where $C_m$ is the minimum detectable concentration and $S_m$ is the signal obtained at that concentration. If the recovery in the sample preparation step is factored in, the detection limit is given as

$$C_m = \frac{s_m - s_{\text{bl}}}{mr} \quad (1.16)$$

Once again, a low recovery increases the detection limit, and a sample preparation technique should aim at 100% recovery.
1.3.3. Range of Quantitation

The lowest concentration level at which a measurement is quantitatively meaningful is called the limit of quantitation (LOQ). The LOQ is most often defined as 10 times the signal/noise ratio. If the noise is approximated as the standard deviation of the blank, the LOQ is $(10 \times s_{bl})$. Once again, when the recovery of the sample preparation step is factored in, the LOQ of the overall method increases by $1/r$.

For all practical purposes, the upper limit of quantitation is the point where the calibration curve becomes nonlinear. This point is called the limit of linearity (LOL). These can be seen from the calibration curve presented in Figure 1.3. Analytical methods are expected to have a linear dynamic range (LDR) of at least two orders of magnitude, although shorter ranges are also acceptable.

Considering all these, the recovery in sample preparation method is an important parameter that affects quantitative issues such as detection limit, sensitivity, LOQ, and even the LOL. Sample preparation techniques that enhance performance (see Chapters 6, 9, and 10) result in a recovery $(r)$ larger than 1, thus increasing the sensitivity and lowering detection limits.

1.3.4. Other Important Parameters

There are several other factors that are important when it comes to the selection of equipment in a measurement process. These parameters are items 7 to 13 in Table 1.2. They may be more relevant in sample preparation than in analysis. As mentioned before, very often the bottleneck is the sample preparation rather than the analysis. The former tends to be slower; consequently, both measurement speed and sample throughput are determined by the discrete steps within the sample preparation. Modern analytical instruments tend to have a high degree of automation in terms of autoinjectors, autosamplers, and automated control/data acquisition. On the other hand, many sample preparation methods continue to be labor-intensive, requiring manual intervention. This prolongs analysis time and introduces random/systematic errors.

A variety of portable instruments have been developed in the last decade. Corresponding sample preparation, or online sample preparation methods, are being developed to make integrated total analytical systems. Many sample preparation methods, especially those requiring extraction, require solvents and other chemicals. Used reagents end up as toxic wastes, whose disposal is expensive. Greener sample preparation methods generate less spent reagent. Last but not the least, cost, including the cost of equipment, labor, and consumables and supplies, is an important factor.
1.3.5. Method Validation

Before a new analytical method or sample preparation technique is to be implemented, it must be validated. The various figures of merit need to be determined during the validation process. Random and systematic errors are measured in terms of precision and bias. The detection limit is established for each analyte. The accuracy and precision are determined at the concentration range where the method is to be used. The linear dynamic range is established and the calibration sensitivity is measured. In general, method validation provides a comprehensive picture of the merits of a new method and provides a basis for comparison with existing methods.

A typical validation process involves one or more of the following steps:

- **Determination of the single operator figures of merit.** Accuracy, precision, detection limits, linear dynamic range, and sensitivity are determined. Analysis is performed at different concentrations using standards.

- **Analysis of unknown samples.** This step involves the analysis of samples whose concentrations are unknown. Both qualitative and quantitative measurements should be performed. Reliable unknown samples are obtained from commercial sources or governmental agencies as certified reference materials. The accuracy and precision are determined.

- **Equivalency testing.** Once the method has been developed, it is compared to similar existing methods. Statistical tests are used to determine if the new and established methods give equivalent results. Typical tests include Student’s t-test for a comparison of the means and the F-test for a comparison of variances.

- **Collaborative testing.** Once the method has been validated in one laboratory, it may be subjected to collaborative testing. Here, identical test samples and operating procedures are distributed to several laboratories. The results are analyzed statistically to determine bias and interlaboratory variability. This step determines the ruggedness of the method.

Method validation depends on the type and purpose of analysis. For example, the recommended validation procedure for PCR, followed by capillary gel electrophoresis of recombinant DNA, may consist of the following steps:

1. Compare precision by analyzing multiple (say, six) independent replicates of reference standards under identical conditions.

2. Data should be analyzed with a coefficient of variation less than a specified value (say, 10%).
3. Validation should be performed on three separate days to compare precision by analyzing three replicates of reference standards under identical conditions (once again the acceptance criteria should be a prespecified coefficient of variation).

4. To demonstrate that other analysts can perform the experiment with similar precision, two separate analysts should make three independent measurements (the acceptance criterion is once again a prespecified RSD).

5. The limit of detection, limit of quantitation, and linear dynamic range are to be determined by serial dilution of a sample. Three replicate measurements at each level are recommended, and the acceptance criterion for calibration linearity should be a prespecified correlation coefficient (say, an $r^2$ value of 0.995 or greater).

6. The molecular weight markers should fall within established migration time ranges for the analysis to be acceptable. If the markers are outside this range, the gel electrophoresis run must be repeated.

### 1.4. PRESERVATION OF SAMPLES

The sample must be representative of the object under investigation. Physical, chemical, and biological processes may be involved in changing the composition of a sample after it is collected. Physical processes that may degrade a sample are volatilization, diffusion, and adsorption on surfaces. Possible chemical changes include photochemical reactions, oxidation, and precipitation. Biological processes include biodegradation and enzymatic reactions. Once again, sample degradation becomes more of an issue at low analyte concentrations and in trace analysis.

The sample collected is exposed to conditions different from the original source. For example, analytes in a groundwater sample that have never been exposed to light can undergo significant photochemical reactions when exposed to sunlight. It is not possible to preserve the integrity of any sample indefinitely. Techniques should aim at preserving the sample at least until the analysis is completed. A practical approach is to run tests to see how long a sample can be held without degradation and then to complete the analysis within that time. Table 1.3 lists some typical preservation methods. These methods keep the sample stable and do not interfere in the analysis.

Common steps in sample preservation are the use of proper containers, temperature control, addition of preservatives, and the observance of recommended sample holding time. The holding time depends on the analyte of interest and the sample matrix. For example, most dissolved metals are
Table 1.3. Sample Preservation Techniques

<table>
<thead>
<tr>
<th>Sample</th>
<th>Preservation Method</th>
<th>Container Type</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>—</td>
<td>—</td>
<td>Immediately on site</td>
</tr>
<tr>
<td>Temperature</td>
<td>—</td>
<td>—</td>
<td>Immediately on site</td>
</tr>
<tr>
<td>Inorganic anions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bromide, chloride, fluoride</td>
<td>None</td>
<td>Plastic or glass</td>
<td>28 days</td>
</tr>
<tr>
<td>Chlorine</td>
<td>None</td>
<td>Plastic or glass</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>Iodide</td>
<td>Cool to 4°C</td>
<td>Plastic or glass</td>
<td>24 hours</td>
</tr>
<tr>
<td>Nitrate, nitrite</td>
<td>Cool to 4°C</td>
<td>Plastic or glass</td>
<td>48 hours</td>
</tr>
<tr>
<td>Sulfide</td>
<td>Cool to 4°C, add zinc acetate and NaOH to pH 9</td>
<td>Plastic or glass</td>
<td>7 days</td>
</tr>
<tr>
<td>Metals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved</td>
<td>Filter on site, acidify to pH 2 with HNO₃</td>
<td>Plastic</td>
<td>6 months</td>
</tr>
<tr>
<td>Total</td>
<td>Acidify to pH 2 with HNO₃</td>
<td>Plastic</td>
<td>6 months</td>
</tr>
<tr>
<td>Cr(VI)</td>
<td>Cool to 4°C</td>
<td>Plastic</td>
<td>24 hours</td>
</tr>
<tr>
<td>Hg</td>
<td>Acidify to pH 2 with HNO₃</td>
<td>Plastic</td>
<td>28 days</td>
</tr>
<tr>
<td>Organics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic carbon</td>
<td>Cool to 4°C, add H₂SO₄ to pH 2</td>
<td>Plastic or brown glass</td>
<td>28 days</td>
</tr>
<tr>
<td>Purgeable hydrocarbons</td>
<td>Cool to 4°C, add 0.008% Na₂S₂O₃</td>
<td>Glass with Teflon septum cap</td>
<td>14 days</td>
</tr>
<tr>
<td>Purgeable aromatics</td>
<td>Cool to 4°C, add 0.008% Na₂S₂O₃ and HCl to pH 2</td>
<td>Glass with Teflon septum cap</td>
<td>14 days</td>
</tr>
<tr>
<td>PCBs</td>
<td>Cool to 4°C</td>
<td>Glass or Teflon</td>
<td>7 days to extraction, 40 days after</td>
</tr>
<tr>
<td>Organics in soil</td>
<td>Cool to 4°C</td>
<td>Glass or Teflon</td>
<td>As soon as possible</td>
</tr>
<tr>
<td>Fish tissues</td>
<td>Freeze</td>
<td>Aluminum foil</td>
<td>As soon as possible</td>
</tr>
<tr>
<td>Biochemical oxygen demand</td>
<td>Cool to 4°C</td>
<td>Plastic or glass</td>
<td>48 hours</td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
<td>Cool to 4°C</td>
<td>Plastic or glass</td>
<td>28 days</td>
</tr>
</tbody>
</table>

(Continued)
stable for months, whereas Cr(VI) is stable for only 24 hours. Holding time can be determined experimentally by making up a spiked sample (or storing an actual sample) and analyzing it at fixed intervals to determine when it begins to degrade.

1.4.1. Volatilization

Analytes with high vapor pressures, such as volatile organics and dissolved gases (e.g., HCN, SO2) can easily be lost by evaporation. Filling sample containers to the brim so that they contain no empty space (headspace) is the most common method of minimizing volatilization. Solid samples can be topped with a liquid to eliminate headspace. The volatiles cannot equilibrate between the sample and the vapor phase (air) at the top of the container. The samples are often held at low temperature (4°C to 8°C) to lower the vapor pressure. Agitation during sample handling should also be avoided. Freezing liquid samples causes phase separation and is not recommended.

1.4.2. Choice of Proper Containers

The surface of the sample container may interact with the analyte. The surfaces can provide catalysts (e.g., metals) for reactions or just sites for irreversible adsorption. For example, metals can adsorb irreversibly on glass surfaces, so plastic containers are chosen for holding water samples to be analyzed for their metal content. These samples are also acidified with HNO3 to help keep the metal ions in solution. Organic molecules may also interact with polymeric container materials. Plasticizers such as phthalate esters can diffuse from the plastic into the sample, and the plastic can serve as a sorbent (or a membrane) for the organic molecules. Consequently, glass containers are suitable for organic analytes. Bottle caps should have Teflon liners to preclude contamination from the plastic caps.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Preservation Method</th>
<th>Container Type</th>
<th>Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Store in TE (pH 8) under ethanol at −20°C; freeze at −20 or −80°C</td>
<td></td>
<td>Years</td>
</tr>
<tr>
<td>RNA</td>
<td>Deionized formamide at −80°C</td>
<td></td>
<td>Years</td>
</tr>
<tr>
<td>Solids unstable in air for surface and spectroscopic characterization</td>
<td>Store in argon-filled box; mix with hydrocarbon oil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.3. (Continued)
Oily materials may adsorb strongly on plastic surfaces, and such samples are usually collected in glass bottles. Oil that remains on the bottle walls should be removed by rinsing with a solvent and be returned to the sample. A sonic probe can be used to emulsify oily samples to form a uniform suspension before removal for analysis.

### 1.4.3. Absorption of Gases from the Atmosphere

Gases from the atmosphere can be absorbed by the sample during handling, for example, when liquids are being poured into containers. Gases such as O₂, CO₂, and volatile organics may dissolve in the samples. Oxygen may oxidize species, such as sulfite or sulfide to sulfate. Absorption of CO₂ may change conductance or pH. This is why pH measurements are always made at the site. CO₂ can also bring about precipitation of some metals. Dissolution of organics may lead to false positives for compounds that were actually absent. Blanks are used to check for contamination during sampling, transport, and laboratory handling.

### 1.4.4. Chemical Changes

A wide range of chemical changes are possible. For inorganic samples, controlling the pH can be useful in preventing chemical reactions. For example, metal ions may oxidize to form insoluble oxides or hydroxides. The sample is often acidified with HNO₃ to a pH below 2, as most nitrates are soluble, and excess nitrate prevents precipitation. Other ions, such as sulfides and cyanides, are also preserved by pH control. Samples collected for NH₃ analysis are acidified with sulfuric acid to stabilize the NH₃ as NH₄SO₄.

Organic species can also undergo changes due to chemical reactions. Storing the sample in amber bottles prevents photooxidation of organics (e.g., polynuclear aromatic hydrocarbons). Organics can also react with dissolved gases; for example, organics can react with trace chlorine to form halogenated compounds in treated drinking water samples. In this case, the addition of sodium thiosulfate can remove the chlorine.

Samples may also contain microorganisms, which may degrade the sample biologically. Extreme pH (high or low) and low temperature can minimize microbial degradation. Adding biocides such as mercuric chloride or pentachlorophenol can also kill the microbes.

### 1.4.5. Preservation of Unstable Solids

Many samples are unstable in air. Examples of air-sensitive compounds are alkali metal intercalated C₆₀, carbon nanotubes, and graphite, which are...
usually prepared in vacuum-sealed tubes. After completion of the intercalation reaction in a furnace, the sealed tubes may be transferred directly to a Raman spectrometer for measurement. Since these compounds are photosensitive, spectra need to be measured using relatively low laser power densities. For x-ray diffraction, infrared, and x-ray photoelectron spectroscopy (XPS), the sealed tubes are transferred to an argon-filled dry box with less than 10 parts per million (ppm) of oxygen. The vacuum tubes are cut open in the dry box and transferred to x-ray sampling capillaries. The open ends of the capillaries are carefully sealed with soft wax to prevent air contamination after removal from the dry box. Samples for infrared spectroscopy are prepared by mixing the solid with hydrocarbon oil and sandwiching a small amount of this suspension between two KBr or NaCl plates. The edges of the plates are then sealed with soft wax. For the XPS measurements, the powder is spread on a tape attached to the sample holder and inserted into a transfer tube of the XPS spectrometer, which had previously been introduced into the dry box. Transfer of unstable compounds into the sampling chamber of transmission and scanning electron microscopes are difficult. The best approaches involve preparing the samples in situ for examination.

1.5. POSTEXTRACTION PROCEDURES

1.5.1. Concentration of Sample Extracts

The analytes are often diluted in the presence of a large volume of solvents used in the extraction. This is particularly true when the analysis is being done at the trace level. An additional concentration step is necessary to increase the concentration in the extract. If the amount of solvent to be removed is not very large and the analyte is nonvolatile, the solvent can be vaporized by a gentle stream of nitrogen gas flowing either across the surface or through the solution. This is shown in Figure 1.6. Care should be taken that the solvent is lost only by evaporation. If small solution droplets are lost as aerosol, there is the possibility of losing analytes along with it. If large volume reduction is needed, this method is not efficient, and a rotary vacuum evaporator is used instead. In this case, the sample is placed in a round-bottomed flask in a heated water bath. A water-cooled condenser is attached at the top, and the flask is rotated continually to expose maximum liquid surface to evaporation. Using a small pump or a water aspirator, the pressure inside the flask is reduced. The mild warming, along with the lowered pressure, removes the solvent efficiently, and the condensed solvent distills into a separate flask. Evaporation should stop before the sample reaches dryness.
For smaller volumes that must be reduced to less than 1 mL, a Kuderna–Danish concentrator (Figure 1.7) is used. The sample is gently heated in a water bath until the needed volume is reached. An air-cooled condenser provides reflux. The volume of the sample can readily be measured in the narrow tube at the bottom.

1.5.2. Sample Cleanup

Sample cleanup is particularly important for analytical separations such as GC, HPLC, and electrophoresis. Many solid matrices, such as soil, can contain hundreds of compounds. These produce complex chromatograms, where the identification of analytes of interest becomes difficult. This is especially true if the analyte is present at a much lower concentration than the interfering species. So a cleanup step is necessary prior to the analytical measurements. Another important issue is the removal of high-boiling materials that can cause a variety of problems. These include analyte adsorption in the injection port or in front of a GC-HPLC column, false positives from interferences that fall within the retention window of the analyte, and false negatives because of a shift in the retention time window.

Figure 1.6. Evaporation of solvent by nitrogen.
In extreme cases, instrument shut down may be necessary due to the accumulation of interfacing species.

Complex matrices such as, soil, biological materials, and natural products often require some degree of cleanup. Highly contaminated extracts (e.g., soil containing oil residuals) may require multiple cleanup steps. On the other hand, drinking water samples are relatively cleaner (as many large molecules either precipitate out or do not dissolve in it) and may not require cleanup [5].

The following techniques are used for cleanup and purification of extracts.

*Gel-Permeation Chromatography*

Gel-permeation chromatography (GPC) is a size-exclusion method that uses organic solvents (or buffers) and porous gels for the separation of macromolecules. The packing gel is characterized by pore size and exclusion range, which must be larger than the analytes of interest. GPC is recommended for the elimination of lipids, proteins, polymers, copolymers, natural resins, cellular components, viruses, steroids, and dispersed high-molecular-weight compounds from the sample. This method is appropriate for both polar and nonpolar analytes. Therefore, it is used for extracts containing a broad range
of analytes. Usually, GPC is most efficient for removing high-boiling mate-
rials that condense in the injection port of a GC or the front of the GC col-
umn [6]. The use of GPC in nucleic acid isolation is discussed in Chapter 8.

**Acid–Base Partition Cleanup**

Acid–base partition cleanup is a liquid–liquid extraction procedure for the
separation of acid analytes, such as organic acids and phenols from base/
neutral analytes (amines, aromatic hydrocarbons, halogenated organic
compounds) using pH adjustment. This method is used for the cleanup of
petroleum waste prior to analysis or further cleanup. The extract from
the prior solvent extraction is shaken with water that is strongly basic. The
basic and neutral components stay in the organic solvent, whereas the acid
analytes partition into the aqueous phase. The organic phase is concentrated
and is ready for further cleanup or analysis. The aqueous phase is acidi-
fied and extracted with an organic solvent, which is then concentrated (if
needed) and is ready for analysis of the acid analytes (Figure 1.8).

**Solid-Phase Extraction and Column Chromatography**

The solvent extracts can be cleaned up by traditional column chroma-
tography or by solid-phase extraction cartridges. This is a common cleanup
method that is widely used in biological, clinical, and environmental sample
preparation. More details are presented in Chapter 2. Some examples
include the cleanup of pesticide residues and chlorinated hydrocarbons, the
separation of nitrogen compounds from hydrocarbons, the separation of
aromatic compounds from an aliphatic–aromatic mixture, and similar
applications for use with fats, oils, and waxes. This approach provides ef-
cient cleanup of steroids, esters, ketones, glycerides, alkaloids, and carbohy-
drates as well. Cations, anions, metals, and inorganic compounds are also
candidates for this method [7].

The column is packed with the required amount of a sorbent and loaded
with the sample extract. Elution of the analytes is effected with a suitable
solvent, leaving the interfering compounds on the column. The packing
material may be an inorganic substance such as Florisil (basic magnesium
silicate) or one of many commercially available SPE stationary phases. The
eluate may be further concentrated if necessary. A Florisil column is shown
in Figure 1.9. Anhydrous sodium sulfate is used to dry the sample [8].

These cleanup and concentration techniques may be used individually, or
in various combinations, depending on the nature of the extract and the
analytical method used.
1.6. QUALITY ASSURANCE AND QUALITY CONTROL DURING SAMPLE PREPARATION

As mentioned earlier, the complete analytical process involves sampling, sample preservation, sample preparation, and finally, analysis. The purpose of quality assurance (QA) and quality control (QC) is to monitor, measure, and keep the systematic and random errors under control. QA/QC measures are necessary during sampling, sample preparation, and analysis. It has been stated that sample preparation is usually the major source of variability in a measurement process. Consequently, the QA/QC during this step is of utmost importance. The discussion here centers on QC during sample preparation.
Quality assurance refers to activities that demonstrate that a certain quality standard is being met. This includes the management process that implements and documents effective QC. Quality control refers to procedures that lead to statistical control of the different steps in the measurement process. So QC includes specific activities such as analyzing replicates, ensuring adequate extraction efficiency, and contamination control.

Some basic components of a QC system are shown in Figure 1.10. Competent personnel and adequate facilities are the most basic QC requirements. Many modern analytical/sample preparation techniques use sophisticated instruments that require specialized training. Good laboratory practice (GLP) refers to the practices and procedures involved in running a laboratory. Efficient sample handling and management, record keeping, and equipment maintenance fall under this category. Good measurement practices (GMPs) refer to the specific techniques in sample preparation and analysis. On the other hand, GLPs are independent of the specific techniques and refer to general practices in the laboratory. An important QC step is to have formally documented GLPs and GMPs that are followed carefully.
Standard operating procedures (SOPs) are written descriptions of procedures of methods being followed. The importance of SOPs cannot be understated when it comes to methods being transferred to other operators or laboratories. Strict adherence to the SOPs reduces bias and improves precision. This is particularly true in sample preparation, which tends to consist of repetitive processes that can be carried out by more than one procedure. For example, extraction efficiency depends on solvent composition, extraction time, temperature, and even the rate of agitation. All these parameters need to be controlled to reduce variability in measurement. Changing the extraction time will change the extraction efficiency, which will increase the relative standard deviation (lower precision). The SOP specifies these parameters. They can come in the form of published standard methods obtained from the literature, or they may be developed in-house. Major sources of SOPs are protocols obtained from organizations, such as the American Society for Testing and Materials and the U.S. Environmental Protection Agency (EPA).

Finally, there is the need for proper documentation, which can be in written or electronic forms. These should cover every step of the measurement process. The sample information (source, batch number, date), sample preparation/analytical methodology (measurements at every step of the process, volumes involved, readings of temperature, etc.), calibration curves, instrument outputs, and data analysis (quantitative calculations, statistical analysis) should all be recorded. Additional QC procedures, such as blanks, matrix recovery, and control charts, also need to be a part of the record keeping. Good documentation is vital to prove the validity of data. Analyt-
ical data that need to be submitted to regulatory agencies also require detailed documentation of the various QC steps.

The major quality parameters to be addressed during sample preparation are listed in Table 1.4. These are accuracy, precision, extraction efficiency (or recovery), and contamination control. These quality issues also need to be addressed during the analysis that follows sample preparation. Accuracy is determined by the analysis of evaluation samples. Samples of known concentrations are analyzed to demonstrate that quantitative results are close to the true value. The precision is measured by running replicates. When many samples are to be analyzed, the precision needs to be checked periodically to ensure the stability of the process. Contamination is a serious issue, especially in trace measurements such as environmental analysis. The running of various blanks ensures that contamination has not occurred at any step, or that if it has, where it occurred. As mentioned before, the detection limits, sensitivity, and other important parameters depend on the recovery. The efficiency of sample preparation steps such as extraction and cleanup must be checked to ensure that the analytes are being recovered from the sample.

### 1.6.1. Determination of Accuracy and Precision

The levels of accuracy and precision determine the quality of a measurement. The data are as good as random numbers if these parameters are not specified. Accuracy is determined by analyzing samples of known concentration (evaluation samples) and comparing the measured values to the known. Standard reference materials are available from regulatory agencies and commercial vendors. A standard of known concentration may also be made up in the laboratory to serve as an evaluation sample.

Effective use of evaluation samples depends on matching the standards with the real-world samples, especially in terms of their matrix. Take the example of extraction of pesticides from fish liver. In a real sample, the pesticide is embedded in the liver cells (intracellular matter). If the calibration standards are made by spiking livers, it is possible that the pesticides will be absorbed on the outside of the cells (extracellular). The extraction of

<table>
<thead>
<tr>
<th>QC Parameters</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>Analysis of reference materials or known standards</td>
</tr>
<tr>
<td>Precision</td>
<td>Analysis of replicate samples</td>
</tr>
<tr>
<td>Extraction efficiency</td>
<td>Analysis of matrix spikes</td>
</tr>
<tr>
<td>Contamination</td>
<td>Analysis of blanks</td>
</tr>
</tbody>
</table>
extracellular pesticides is easier than real-world intracellular extractions. Consequently, the extraction efficiency of the spiked sample may be significantly higher. Using this as the calibration standard may result in a negative bias. So matrix effects and matrix matching are important for obtaining high accuracy. Extraction procedures that are powerful enough not to have any matrix dependency are desirable.

Precision is measured by making replicate measurements. As mentioned before, it is known to be a function of concentration and should be determined at the concentration level of interest. The intrasample variance can be determined by splitting a sample into several subsamples and carrying out the sample preparation/analysis under identical conditions to obtain a measure of RSD. For example, several aliquots of homogenized fish liver can be processed through the same extraction and analytical procedure, and the RSD computed. The intersample variance can be measured by analyzing several samples from the same source. For example, different fish from the same pond can be analyzed to estimate the intersample RSD.

The precision of the overall process is often determined by the extraction step rather than the analytical step. It is easier to get high-precision analytical results; it is much more difficult to get reproducible extractions. For example, it is possible to run replicate chromatographic runs (GC or HPLC) with an RSD between 1 and 3%. However, several EPA-approved methods accept extraction efficiencies anywhere between 70 and 120%. This range alone represents variability as high as 75%. Consequently, in complex analytical methods that involve several preparative steps, the major contributor to variability is the sample preparation.

1.6.2. Statistical Control

Statistical evidence that the precision of the measurement process is within a certain specified limit is referred to as statistical control. Statistical control does not take the accuracy into account. However, the precision of the measurement should be established and statistical control achieved before accuracy can be estimated.

Control Charts

Control charts are used for monitoring the variability and to provide a graphical display of statistical control. A standard, a reference material of known concentration, is analyzed at specified intervals (e.g., every 50 samples). The result should fall within a specified limit, as these are replicates. The only variation should be from random error. These results are plotted on a control chart to ensure that the random error is not increasing or that a
systematic bias is not taking place. In the control chart shown in Figure 1.11, replicate measurements are plotted as a function of time. The centerline is the average, or expected value. The upper (UCL) and lower (LCL) control limits are the values within which the measurements must fall. Normally, the control limits are \( \pm 3\sigma \), within which 99.7% of the data should lie. For example, in a laboratory carrying out microwave extraction on a daily basis, a standard reference material is extracted after a fixed number of samples. The measured value is plotted on the control chart. If it falls outside the control limit, readjustments are necessary to ensure that the process stays under control.

Control charts are used in many different applications besides analytical measurements. For example, in a manufacturing process, the control limits are often based on product quality. In analytical measurements, the control limits can be established based on the analyst’s judgment and the experimental results. A common approach is to use the mean of select measurements as the centerline, and then a multiple of the standard deviation is used to set the control limits. Control charts often plot regularly scheduled analysis of a standard reference material or an audit sample. These are then tracked to see if there is a trend or a systematic deviation from the centerline.
Control Samples

Different types of control samples are necessary to determine whether a measurement process is under statistical control. Some of the commonly used control standards are listed here.

1. **Laboratory control standards** (LCSs) are certified standards obtained from an outside agency or commercial source to check whether the data being generated are comparable to those obtained elsewhere. The LCSs provide a measure of the accuracy and can be used as audits. A source of LCSs is **standard reference materials** (SRMs), which are certified standards available from the National Institute of Standards and Testing (NIST) in the United States. NIST provides a variety of solid, liquid, and gaseous SRMs which have been prepared to be stable and homogeneous. They are analyzed by more than one independent methods, and their concentrations are certified. Certified standards are also available from the European Union Community Bureau of Reference (BCR), government agencies such as the EPA, and from various companies that sell standards. These can be quite expensive. Often, samples are prepared in the laboratory, compared to the certified standards, and then used as secondary reference materials for daily use.

2. **Calibration control standards** (CCSs) are used to check calibration. The CCS is the first sample analyzed after calibration. Its concentration may or may not be known, but it is used for successive comparisons. A CCS may be analyzed periodically or after a specified number of samples (say, 20). The CCS value can be plotted on a control chart to monitor statistical control.

1.6.3. Matrix Control

**Matrix Spike**

Matrix effects play an important role in the accuracy and precision of a measurement. Sample preparation steps are often sensitive to the matrix. Matrix spikes are used to determine their effect on sample preparation and analysis. Matrix spiking is done by adding a known quantity of a component that is similar to the analyte but not present in the sample originally. The sample is then analyzed for the presence of the spiked material to evaluate the matrix effects. It is important to be certain that the extraction recovers most of the analytes, and spike recovery is usually required to be at least 70%. The matrix spike can be used to accept or reject a method.
For example, in the analysis of chlorophenol in soil by accelerated solvent extraction followed by GC-MS, deuterated benzene may be used as the matrix spike. The deuterated compound will not be present in the original sample and can easily be identified by GC-MS. At the same time, it has chemical and physical properties that closely match those of the analyte of interest.

Often, the matrix spike cannot be carried out at the same time as the analysis. The spiking is carried out separately on either the same matrix or on one that resembles the samples. In the example above, clean soil can be spiked with regular chlorophenol and then the recovery is measured. However, one should be careful in choosing the matrix to be spiked. For instance, it is easy to extract different analytes from sand, but not so if the analytes have been sitting in clay soil for many years. The organics in the soil may provide additional binding for the analytes. Consequently, a matrix spike may be extracted more easily than the analytes in real-world samples. The extraction spike may produce quantitative recovery, whereas the extraction efficiency for real samples may be significantly lower. This is especially true for matrix-sensitive techniques, such as supercritical extraction.

**Surrogate Spike**

Surrogate spikes are used in organic analysis to determine if an analysis has gone wrong. They are compounds that are similar in chemical composition and have similar behavior during sample preparation and analysis. For example, a deuterated analog of the analyte is an ideal surrogate during GC-MS analysis. It behaves like the analyte and will not be present in the sample originally. The surrogate spike is added to the samples, the standards, the blanks, and the matrix spike. The surrogate recovery is computed for each run. Unusually high or low recovery indicates a problem, such as contamination or instrument malfunction. For example, consider a set of samples to be analyzed for gasoline contamination by purge and trap. Deuterated toluene is added as a surrogate to all the samples, standards, and blanks. The recovery of the deuterated toluene in each is checked. If the recovery in a certain situation is unusually high or low, that particular analysis is rejected.

**1.6.4. Contamination Control**

Many measurement processes are prone to contamination, which can occur at any point in the sampling, sample preparation, or analysis. It can occur in the field during sample collection, during transportation, during storage, in the sample workup prior to measurement, or in the instrument itself. Some
common sources of contamination are listed in Table 1.5. Contamination becomes a major issue in trace analysis. The lower the concentration, the more pronounced is the effect of contamination.

Sampling devices themselves can be a source of contamination. Contamination may come from the material of construction or from improper cleaning. For example, polymer additives can leach out of plastic sample bottles, and organic solvents can dissolve materials from surfaces, such as cap liners of sample vials. Carryover from previous samples is also possible. Say that a sampling device was used where the analyte concentration was at the 1 ppm level. A 0.1% carryover represents a 100% error if the concentration of the next sample is at 1 part per billion (ppb).

Contamination can occur in the laboratory at any stage of sample preparation and analysis. It can come from containers and reagents or from the ambient environment itself. In general, contamination can be reduced by avoiding manual sample handling and by reducing the number of discrete processing steps. Sample preparations that involve many unautomated manual steps are prone to contamination. Contaminating sources can also be present in the instrument. For instance, the leftover compounds from a previous analysis can contaminate incoming samples.

### Blanks

Blanks are used to assess the degree of contamination in any step of the measurement process. They may also be used to correct relatively constant,
unavoidable contamination. Blanks are samples that do not contain any (or a negligible amount of) analyte. They are made to simulate the sample matrix as closely as possible. Different types of blanks are used, depending on the procedure and the measurement objectives. Some common blanks are listed in Table 1.6. Blank samples from the laboratory and the field are required to cover all the possible sources of contamination. We focus here on those blanks that are important from a sample preparation perspective.

**System or Instrument Blank.** It is a measure of system contamination and is the instrumental response in the absence of any sample. When the background signal is constant and measurable, the usual practice is to consider that level to be the zero setting. It is generally used for analytical instruments but is also applicable for instruments for sample preparation.

### Table 1.6. Types of Blanks

<table>
<thead>
<tr>
<th>Blank Type</th>
<th>Purpose</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>System or instrument blank</td>
<td>Establishes the baseline of an instrument in the absence of sample</td>
<td>Determine the background signal with no sample present</td>
</tr>
<tr>
<td>Solvent or calibration blank</td>
<td>To measure the amount of the analytical signal that arises from the solvents and reagents; the zero solution in the calibration series</td>
<td>Analytical instrument is run with solvents/reagents only</td>
</tr>
<tr>
<td>Method blank</td>
<td>To detect contamination from reagents, sample handling, and the entire measurement process</td>
<td>A blank is taken through the entire measurement procedure</td>
</tr>
<tr>
<td>Matched-matrix blank</td>
<td>To detect contamination from field handling, transportation, or storage</td>
<td>A synthetic sample that matches the basic matrix of the sample is carried to the field and is treated in the same fashion as the sample</td>
</tr>
<tr>
<td>Sampling media</td>
<td>To detect contamination in the sampling media such as filters and sample adsorbent traps</td>
<td>Analyze samples of unused filters or traps to detect contaminated batches</td>
</tr>
<tr>
<td>Equipment blank</td>
<td>To determine contamination of equipment and assess the efficiency or equipment cleanup procedures</td>
<td>Samples of final equipment cleaning rinses are analyzed for contaminants</td>
</tr>
</tbody>
</table>
The instrument blank also identifies memory effects or carryover from previous samples. It may become significant when a low-concentration sample is analyzed immediately after a high-concentration sample. This is especially true where preconcentration and cryogenic steps are involved. For example, during the purge and trap analysis of volatile organics, some components may be left behind in the sorbent trap or at a cold spot in the instrument. So it is a common practice to run a deionized water blank between samples. These blanks are critical in any instrument, where sample components may be left behind only to emerge during the next analysis.

**Solvent/Reagent Blank.** A solvent blank checks solvents and reagents that are used during sample preparation and analysis. Sometimes, a blank correction or zero setting is done based on the reagent measurement. For example, in atomic or molecular spectroscopy, the solvents and reagents used in sample preparation are used to provide the zero setting.

**Method Blank.** A method blank is carried through all the steps of sample preparation and analysis as if it were an actual sample. This is most important from the sample preparation prospective. The same solvents/reagents that are used with the actual samples are used here. For example, in the analysis of metals in soil, a clean soil sample may serve as a method blank. It is put through the extraction, concentration, and analysis steps encountered by the real samples. The method blank accounts for contamination that may occur during sample preparation and analysis. These could arise from the reagents, the glassware, or the laboratory environment.

Other types of blanks may be employed as the situation demands. It should be noted that blanks are effective only in identifying contamination. They do not account for various errors that might exist. Blanks are seldom used to correct for contamination. More often, a blank above a predetermined value is used to reject analytical data, making reanalysis and even resampling necessary. The laboratory SOPs should identify the blanks necessary for contamination control.

**REFERENCES**


