Chapter 8

Sampling, standardization, and Calibration
- Sampling is one of the most important operations in a chemical analysis.

- Chemical analyses use only a small fraction of the available sample. The fractions of the samples that collected for analyses must be representative of the bulk materials.

- Knowing how much sample to collect and how to further subdivide the collected sample to obtain a laboratory sample is vital in the analytical process.

- All three steps of sampling, standardization, and calibration require a knowledge of statistics.
## 8A Analytical samples and methods

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Type of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 0.1g</td>
<td>Macro</td>
</tr>
<tr>
<td>0.01~0.1g</td>
<td>Semimicro</td>
</tr>
<tr>
<td>0.0001~0.01g</td>
<td>Micro</td>
</tr>
<tr>
<td>&lt; 10^-4 g</td>
<td>Ultramicro</td>
</tr>
</tbody>
</table>

Figure 8-1 Classification of analyses by sample size.

<table>
<thead>
<tr>
<th>Analytical level</th>
<th>Type of constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%~100%</td>
<td>Major</td>
</tr>
<tr>
<td>0.01%(100ppm)~1%</td>
<td>Minor</td>
</tr>
<tr>
<td>1ppb~100ppm</td>
<td>Trace</td>
</tr>
<tr>
<td>&lt;1 ppb</td>
<td>Ultratrace</td>
</tr>
</tbody>
</table>

Figure 8-2 Classification of constituent types by analyte level.
Figure 8-3  Interlaboratory error as a function of analyte concentration. Note that the relative standard deviation dramatically increase as the analyte concentration decreases.

Real Samples

Matrix is the medium containing analyte.

A **matrix effect** is a change in the analytical signal caused by anything in the sample other than analyte.

Sample are **analyzed**, but species (constituents) or concentrations are **determined**.
8B **Sampling** is the process by which a sample population is reduced in size to an amount of **homogeneous** material that can be conveniently handled in the lab and whose composition is **representative of the population** (unbiased estimate of population mean).

Ex.

Population: 100 coins

Each coin is a sampling unit or an increment

Gross sample: 5 coins

the collection of individual sampling units or increments

Lab sample: the gross sample is reduced in size and made homogeneous
Fig. 8-4  Steps in obtaining a lab sample (a few grams ~ a few hundred grams). Lab sample may constitute as little as 1 part in $10^7$ or $10^8$ of the bulk material.
QUARTERING SAMPLES

A method of obtaining a representative sample for analysis or test of an aggregate with occasional shovelsful, of which a heap or cone is formed, This is flattened out and two opposite quarter parts are rejected. Another cone is formed from the remainder which is again quartered, the process being repeated until a sample of the required size is left.

The procedures vary somewhat, depending upon the size of the sample.

Quartering Method

Mix samples thoroughly. Pour it onto a large flat surface.

Divide the sample into four equal parts.

Save the 2 opposite quarters. Be sure to save the fine material at the bottom of the saved quarter. If the sample is still to large, divide the sample again.
Sampling

Sampling is the process of extracting from a large quantity of material a small portion, which is truly representative of the composition of the whole material.

1) *Three main group of sampling:*

   1> **Census**: all the material is examined ⇒ impracticable
   
   2> **Casual sampling** on an *ad hoc* basis ⇒ unscientific

   3> **Statistical sampling**

2) *Sampling procedure*
Census vs Random sampling

Census

A complete enumeration, usually of a population, but also businesses and commercial establishments, farms, governments, and so forth.

A complete study of the population as compared to a sample.

Random sampling

A commonly used sampling technique in which sample units are selected so that all combinations of $n$ units under consideration have an equal chance of being selected as the sample.

(Statistical sampling meaning) A sampling method in which every possible sample has the same chance of being selected.
3) **Sampling statistics**:

Overall standard deviation \((s_0)\) = sampling error + analytical method error

\[ s_0 = (s_S^2 + s_m^2) \]

In designing a sampling plan the following points should be considered.

1> the number of samples to be taken
2> the size of the sample
3> should individual samples be analyzed or should a sample composed of two or more increments (composite) be prepared.
How much should be analyzed?

\[ mR^2 \approx K_S \]

where \( m \) = mass of each sample analyzed, \( R \) = desired relative SD.

How many portions should be analyzed?

\[ e = \left( ts_S \right) / \left( n \right)^{1/2} \]
\[ \therefore n = t^2 s_S^2 / e^2 \]

where \( n \) = the number of samples needed

\[ t = \text{Student’s t for the 95% confidence level and } n-1 \]

degree of freedom.

Statistics of sampling segregated materials

\[ s_S^2 = [A / mn] + [B / n] \]

where \( s_S \) is the standard deviation of \( n \) samples, each of mass \( m \).

The constant \( A \) and \( B \) are properties of the bulk material and must be measured in preliminary experiments.
### Sampling uncertainties

Both systematic and random errors in analytical data can be traced to instrument, method, and personal causes. Most systematic errors can be eliminated by exercising care, by calibration, and by the proper use of standards, blanks, and reference materials.

For random and independent uncertainties, the overall standard deviation $s_0$ for an analytical measurement is related to the standard deviation of the sampling process $s_s$ and the standard deviation of the method $s_m$ by the relationship

$$s_0^2 = s_s^2 + s_m^2$$

When $s_m < s_s/3$, there is no point in trying to improve the measurement precision.
Size of the gross sample

The gross sample is the collection of individual sampling units. It must be representative of the whole in composition and in particle-size distribution.

Basically, gross sample weight is determined by

(1) The uncertainty that can be tolerated between the composition of the gross sample and that of the whole,

(2) The degree of heterogeneity of the whole,

(3) The level of particle size at which heterogeneity begins.

To obtain a truly representative gross sample, a certain number $N$ of particles must be taken. The number of particles required in a gross sample ranges from a few particles to $10^{12}$ particles.
Sampling homogeneous solutions of liquids and gases

Well mixed solutions of liquids and gases require only a very small sample because they are homogeneous down to the molecular level.

Homogeneous?
Which portion?
Flowing stream?

Gas sampling: Sampling bag (Tedlar® bag)

with a Teflon fitting

Trap in a liquid

Adsorbed onto the surface of a solid

SPME
Inhomogeneous liquid, such as a lake, or an inhomogeneous solid, such as an ore, a soil, or a piece of animal tissue.
SAMPLE PREPARATION

(1) Is the sample a Solid or a Liquid?

Liquids

(2) Are you interested in all sample components or only one or a few?
If only a few then separation is necessary by extraction or chromatography.

(3) Is the concentration of the analytes appropriate for the measurement technique?
If not, dilute or concentrate with extraction, evaporation, lyophilization.

(4) Is sample unstable?
If yes, derivatize, cool, freeze, store in dark

(5) Is the liquid or solvent compatible with the analytical method?
If not, do solvent exchange with extraction, distillation, lyophilization.

http://www.trincoll.edu/~dhenders/textfi~1/Chem%20208%20notes/sample_preparation.htm
As an idealized example, let us presume that a pharmaceutical mixture contains just two types of particles: type A particles containing the active ingredient and type B particles containing only an inactive filler material. All particles are the same size. We wish to collect a gross sample that will allow us to determine the percentage of particles containing the active ingredient in the bulk material.

Assume that the probability of randomly drawing an A type particle is $p$ and that of randomly drawing a B type particle is $(1 - p)$. If $N$ particles of the mixture are taken, the most probable value for the number of A type particles is $pN$, while the most probable number of B type particles is $(1 - p)N$. For such a binary population, the Bernoulli equation can be used to calculate the standard deviation of the number of A particles drawn, $\sigma_A$.

$$\sigma_A = \sqrt{Np(1 - p)} \quad (8-2)$$

The relative standard deviation $\sigma_r$ of drawing A type particles is $\sigma_A/Np$.

$$\sigma_r = \frac{\sigma_A}{Np} = \sqrt{\frac{1 - p}{Np}} \quad (8-3)$$

From Equation 8-3, we can obtain the number of particles needed to achieve a given relative standard deviation as shown in Equation 8-4.

$$N = \frac{1 - p}{p \sigma_r^2} \quad (8-4)$$
We can now make the problem more realistic and assume that both of the components in the mixture contain the active ingredient (analyte), although in differing percentages. The type A particles contain a higher percentage of analyte, $P_A$ and the type B particles a lesser amount, $P_B$. Furthermore, the average density $d$ of the particles differs from the densities $d_A$ and $d_B$ of these components. We must now decide what number of particles and thus what mass we should take to ensure that we have a sample with the overall average percent of active ingredient $P$ with a sampling relative standard deviation of $\sigma_r$. Equation 8-4 can be extended to include these conditions:

$$N = p(1 - p) \left( \frac{d_A d_B}{d^2} \right)^2 \left( \frac{P_A - P_B}{\sigma_r P} \right)^2$$  \hspace{1cm} (8-5)

From this equation, we see that the demands of precision are costly in terms of the sample size required because of the inverse-square relationship between the allowable relative standard deviation and the number of particles taken. Also, we can see that a greater number of particles must be taken as the average percentage $P$ of the active ingredient becomes smaller.

The degree of heterogeneity as measured by $P_A - P_B$ has a large influence on the number of particles required since $N$ increases with the square of the difference in composition of the two components of the mixture.

We can rearrange Equation 8-5 to calculate the relative standard deviation of sampling, $\sigma_r$.

$$\sigma_r = \frac{|P_A - P_B|}{P} \times \frac{d_A d_B}{d^2} \sqrt{\frac{p(1 - p)}{N}}$$ \hspace{1cm} (8-6)
We can rearrange Equation 8-5 to calculate the relative standard deviation of sampling, $\sigma_r$.

$$\sigma_r = \frac{|P_A - P_B|}{p} \times \frac{d_A d_B}{d^2} \sqrt{\frac{p(1 - p)}{N}} \quad (8-6)$$

If we make the assumption that the sample mass $m$ is proportional to the number of particles and the other quantities in Equation 8-6 are constant, the product of $m$ and $\sigma_r$ should be a constant. This constant $K_s$ is called the Ingamells sampling constant.\(^6\) Thus,

$$K_s = m \times (\sigma_r \times 100)^2 \quad (8-7)$$

where the term $\sigma_r \times 100\%$ is the percent relative standard deviation. Hence, when $\sigma_r = 0.01$, $\sigma_r \times 100\% = 1\%$, and $K_s$ is just equal to $m$. We can thus interpret the sampling constant $K_s$ to be the minimum sample mass required to reduce the sampling uncertainty to 1%.

The problem of deciding on the mass of the gross sample for a solid substance is usually even more difficult than this example because most materials not only contain more than two components, but they also consist of a range of particle sizes. In most instances, the problem of multiple components can be met by dividing the sample into an imaginary two-component system. Thus, with an actual complex mixture of substances, one component selected might be all the various analyte-containing particles and the other all the residual components containing little or no analyte.
Figure 8-6  Steps in sampling a particulate solids
Number of Laboratory Samples

If the sampling standard deviation $\sigma_s$ is known from previous experience, we can use values of $z$ from tables (see Section 7A-1).

$$\text{CI for } \mu = \bar{x} \pm \frac{z\sigma_s}{\sqrt{N}}$$

Often, we use an estimate of $\sigma_s$ and so must use $t$ instead of $z$ (Section 7A-2)

$$\text{CI for } \mu = \bar{x} \pm \frac{ts_s}{\sqrt{N}}$$

The last term in this equation represents the absolute uncertainty that we can tolerate at a particular confidence level. If we divide this term by the mean value $\bar{x}$, we can calculate the relative uncertainty $\sigma_r$ that is tolerable at a given confidence level:

$$\sigma_r = \frac{ts_s}{\bar{x}\sqrt{N}} \quad \text{(8-8)}$$

If we solve Equation 8-8 for the number of samples $N$, we obtain

$$N = \frac{t^2s_s^2}{\bar{x}^2\sigma_r^2} \quad \text{(8-9)}$$
Automated Sample handling

Automated sample handling can lead to higher throughput (more analyses per unit time), higher reliability, and lower costs than manual sample handling.

Discrete (or batch) methods

Continuous Flow methods
  segmented flow analyzer
  flow injection analyzer (FIA)
Figure 8-7 Segmented continuous flow analyzer. Samples are aspirated from sample cups in the sampler and pumped into the manifold where they are mixed with one or more reagents. Air is also injected to segment the samples with bubbles. The bubbles are usually removed by a debubbler before the stream reaches the detector. The segmented sample is shown in more detail in (b). The bubbles minimize dispersion of the sample that can cause broadening of the zones and crosscontamination from different samples. The analyte concentration profiles at the sampler and at the detector are shown in (c). Normally the height of a sample peak is related to the concentration of the analyte.
Figure 8-8 Flow injection analyzer. In (a) the sample is loaded from a sampler into the sample loop of a sampling valve. The valve, shown in the load position, also has a second inject position shown by the dotted lines. When switched to the inject position, the stream containing the reagent flows through the sample loop. Sample and reagent are allowed to mix and react in the mixing coil before reaching the detector. In this case, the sample plug is allowed to disperse prior to reaching the detector (b). The resulting concentration profile (detector response) depends on the degree of dispersion.
Microfluidic systems in which operations are miniaturized to the scale of an integrated circuit has enabled the fabrication of a complete laboratory-on-a-chip or micro total analysis system (μTAS). Fig. 8F1 shows the layout of a microstructure used for FIA. The monolithic unit is made of two polydimethyl siloxane (PDMS) layers that are permanently bonded together. The fluidic channels are 100 mm wide and 10 mm high. The entire device is only 2.0 cm by 2.0 cm. A glass cover allows for optical imaging of the channels by fluorescence excited with an Ar ion laser.

Layout of a microfabricated structure for FIA. Microfluidic channels are shown in blue, while control channels (pumps and valves) are shown in black. The components are (a) peristaltic pump, (b), injection valve, (c), mixing/reaction chamber, and (d), sample selector. Blue circles represent fluid reservoirs. Numbers (1) and (2) are samples, (3) is the carrier, (4) the reagent, and (5) and (6) are waste reservoirs. The entire structure is 2.0 cm by 2.0 cm.
A Comparison Method for Aflatoxins

In the comparison method, antibodies specific to the aflatoxin are coated on the base of a plastic compartment or microtiter well in an array on a plate such as that shown in Figure 8F-2. The aflatoxin behaves as the antigen. During the analysis, an enzyme reaction causes a blue product to be formed. As the amount of aflatoxin in the sample increases, the blue color decreases in intensity. The color-measuring instrument is the basic fiber optic comparator shown in Figure 8F-3.
8D Standardization and Calibration

Calibration determines the relationship between the analytical response and the analyte concentration. This relationship is usually determined by the use of chemical standards. The standards used can be prepared from purified reagents, if available, or standardized by classical quantitative methods (see Chapters 12–17). Most commonly, the standards used are prepared externally to the analyte solutions (external standard methods). In some cases, an attempt is made to reduce interferences from other constituents in the sample matrix, called concomitants, by using standards added to the analyte solution (internal standard methods or standard addition methods) or by matrix matching or modification.

direct comparison techniques ----- **null comparison** or **isolation methods**

titration procedures ---- In a titration, the analyte reacts with a standardized reagent (the titrant) in a known stoichiometric manner. Usually the amount of titrant is varied until chemical equivalence is reached as indicated by the color change of a chemical indicator or by the change in an instrument response.
External Standard Calibration

In external standard calibration, a series of standard solutions is prepared separately from the sample. The standards are used to establish the instrument calibration function, which is obtained from analysis of the instrument response as a function of the known analyte concentration. Ideally, three or more standard solutions are used in the calibration process, although in some routine determinations, two-point calibrations can be reliable.

The calibration function can be obtained graphically or in mathematical form. Generally, a plot of instrument response versus known analyte concentrations is used to produce a calibration curve, sometimes called a working curve. It is often desirable that the calibration curve be linear in at least the range of the analyte concentrations.
Figure 8-9 Calibration curve of absorbance versus analyte concentration for a series of standards. Data for standards shown as solid circles. The calibration curve is used in an inverse fashion to obtain the concentration of an unknown with an absorbance of 0.505.
Figure 8-10 The slope-intercept form of a straight line.
Linear regression

Linear regression uses the method of least squares to determine the best linear equation to describe a set of $x$ and $y$ data points. The method of least squares minimizes the sum of the square of the residuals - the difference between a measured data point and the hypothetical point on a line. The residuals must be squared so that positive and negative values do not cancel.

$$\text{Residual} = y_i - (mx_i + b)$$

A common application of linear regression in analytical chemistry is to determine the best linear equation for calibration data to generate a calibration or working curve. The concentration of an analyte in a sample can then be determined by comparing a measurement of the unknown to the calibration curve.

**Equation of calibration line:**

$$y(\pm s_y) = [m(\pm s_m)]x + [b(\pm s_b)]$$
Assumptions of the Least Squares Method.

Two assumptions are made in using the method of least squares.

The first is that there is actually a linear relationship between the measured response $y$ (absorbance in Figure 8-9) and the standard analyte concentration $x$. The mathematical relationship that describes this assumption is called the regression model, which may be represented as

$$y = mx + b$$

where $b$ is the y intercept (the value of $y$ when $x$ is zero), and $m$ is the slope of the line (see Figure 8-10).

We also assume that any deviation of the individual points from the straight line arises from error in the measurement. That is, we assume there is no error in $x$ values of the points (concentration).
Different types of best-fitting straight lines. These graphs show the best-fitting straight lines through the same five data points as calculated by minimizing the sum of the squares of the vertical residuals, which assumes that x is the independent variable (A); horizontal residuals, which assumes that y is the independent variable (B); and perpendicular residuals which involves no assumptions about the variables (C).

Tutorial: How to draw the line in biomedical research Lisa Huang, Amir Rattner, Han Liu, Jeremy Nathans
- See more at:http://elifesciences.org/content/2/e00638#.dpuf
Both of these assumptions are appropriate for many analytical methods, but bear in mind that, whenever there is significant uncertainty in the $x$ data, basic linear least-squares analysis may not give the best straight line. In such a case, a more complex correlation analysis may necessary.

In addition, simple least-squares analysis may not be appropriate when the uncertainties in the $y$ values vary significantly with $x$. In that instance, it may be necessary to apply different weighting factors to the points and perform a weighted least-squares analysis.
Finding the Least-Squares Line

The least-squares procedure can be illustrated with the aid of the calibration curve for the determination of Ni(II) shown in Figure 8-9. Thiocyanate was added to the Ni(II) standards, and the absorbances measured as a function of the Ni(II) concentration. The vertical deviation of each point from the straight line is called a residual as shown in the inset. The residuals must be squared so that positive and negative values do not cancel.

\[
\text{Residual} = y_i - (mx_i + b)
\]

The method of least squares minimizes the sum of the square of the residuals - the difference between a measured data point and the hypothetical point on a line. In addition to providing the best fit between the experimental points and the straight line, the method gives the standard deviations for \(m\) and \(b\).

The least-squares method finds the sum of the squares of the residuals \(SS_{\text{resid}}\) and minimizes the sum using calculus.\(^1\) The value of \(SS_{\text{resid}}\) is found from

\[
SS_{\text{resid}} = \sum_{i=1}^{N} [y_i - (b + mx_i)]^2
\]
The equations for \( S_{xx} \) and \( S_{yy} \) are the numerators in the equations for the variance in \( x \) and the variance in \( y \). Likewise, \( S_{xy} \) is the numerator in the covariance of \( x \) and \( y \).

\[
S_{xx} = \sum (x_i - \bar{x})^2 = \sum x_i^2 - \frac{(\sum x_i)^2}{N} \tag{8-10}
\]

\[
S_{yy} = \sum (y_i - \bar{y})^2 = \sum y_i^2 - \frac{(\sum y_i)^2}{N} \tag{8-11}
\]

\[
S_{xy} = \sum (x_i - \bar{x})(y_i - \bar{y}) = \sum x_i y_i - \frac{\sum x_i \sum y_i}{N} \tag{8-12}
\]

where \( x_i \) and \( y_i \) are individual pairs of data for \( x \) and \( y \), \( N \) is the number of pairs, and \( \bar{x} \) and \( \bar{y} \) are the average values for \( x \) and \( y \), that is, \( \bar{x} = \frac{\sum x_i}{N} \), and \( \bar{y} = \frac{\sum y_i}{N} \).

Note that \( S_{xx} \) and \( S_{yy} \) are the sum of the squares of the deviations from the mean for individual values of \( x \) and \( y \). The expressions shown on the far right in Equations 8-10 through 8-12 are more convenient when a calculator without a built-in regression function is used.

The equations for \( S_{xx} \) and \( S_{yy} \) are the numerators in the equations for the variance in \( x \) and the variance in \( y \). Likewise, \( S_{xy} \) is the numerator in the covariance of \( x \) and \( y \).
Six useful quantities can be derived from $S_{xx}$, $S_{yy}$, and $S_{xy}$:

1. The slope of the line, $m$:
   \[ m = \frac{S_{xy}}{S_{xx}} \]  
   (8-13)

2. The intercept, $b$:
   \[ b = \bar{y} - m\bar{x} \]  
   (8-14)

3. The standard deviation about regression, $s_r$:
   \[ s_r = \sqrt{\frac{S_{yy} - m^2 S_{xx}}{N - 2}} \]  
   (8-15)

4. The standard deviation of the slope, $s_m$:
   \[ s_m = \sqrt{\frac{s_r^2}{S_{xx}}} \]  
   (8-16)

5. The standard deviation of the intercept, $s_b$:
   \[ s_b = s_r \sqrt{\frac{\sum x_i^2}{N \sum x_i^2 - (\sum x_i)^2}} = s_r \sqrt{\frac{1}{N - (\sum x_i)^2 / \sum x_i^2}} \]  
   (8-17)

6. The standard deviation for results obtained from the calibration curve, $s_c$:
   \[ s_c = \frac{s_r}{m} \sqrt{\frac{1}{M} + \frac{1}{N} + \frac{(\bar{y}_c - \bar{y})^2}{m^2 S_{xx}}} \]  
   (8-18)
**Example 8-4**

Carry out a least-squares analysis of the calibration data for the determination of isoctane in a hydrocarbon mixture provided in the first two columns of Table 8-1. Columns 3, 4, and 5 of the table contain computed values for $x_i^2$, $y_i^2$, and $x_i y_i$, with their sums appearing as the last entry in each column. Note that the number of digits carried in the computed values should be the *maximum allowed by the calculator or computer*, that is, *rounding should not be performed until the calculation is complete.*
Solution

We now substitute into Equations 8-10, 8-11, and 8-12 and obtain

\[ S_{xx} = \sum x_i^2 - \frac{(\sum x_i)^2}{N} = 6.9021 - \frac{(5.365)^2}{5} = 1.14537 \]

\[ S_{yy} = \sum y_i^2 - \frac{(\sum y_i)^2}{N} = 36.3775 - \frac{(12.51)^2}{5} = 5.07748 \]

\[ S_{xy} = \sum x_i y_i - \frac{\sum x_i \sum y_i}{N} = 15.81992 - \frac{5.365 \times 12.51}{5} = 2.39669 \]

Substitution of these quantities into Equations 8-13 and 8-14 yields

\[ m = \frac{2.39669}{1.14537} = 2.0925 \approx 2.09 \]

\[ b = \frac{12.51}{5} - 2.0925 \times \frac{5.365}{5} = 0.2567 \approx 0.26 \]

Thus, the equation for the least-squares line is

\[ y = 2.09x + 0.26 \]
Substitution into Equation 8-15 yields the standard deviation about regression,

\[
\sigma_r = \sqrt{\frac{S_{yy} - m^2S_{xx}}{N - 2}} = \sqrt{\frac{5.07748 - (2.0925)^2 \times 1.14537}{5 - 2}} = 0.1442 \approx 0.14
\]

and substitution into Equation 8-16 gives the standard deviation of the slope,

\[
\sigma_m = \sqrt{\frac{\sigma_r^2}{S_{xx}}} = \sqrt{\frac{(0.1442)^2}{1.14537}} = 0.13
\]

Finally, we find the standard deviation of the intercept from Equation 8-17:

\[
\sigma_b = 0.1442\sqrt{\frac{1}{5 - (5.365)^2/6.9021}} = 0.16
\]
EXAMPLE 8-5

The calibration curve found in Example 8-4 was used for the chromatographic determination of isoctane in a hydrocarbon mixture. A peak area of 2.65 was obtained. Calculate the mole percent of isoctane in the mixture and the standard deviation if the area was (a) the result of a single measurement and (b) the mean of four measurements.

Solution

In either case, the unknown concentration is found from rearranging the least-squares equation for the line, which gives

\[ x = \frac{y - b}{m} = \frac{y - 0.2567}{2.0925} = \frac{2.65 - 0.2567}{2.0925} = 1.144 \text{ mol }\%
\]

(a) Substituting into Equation 8-18, we obtain

\[ s_c = \frac{0.1442}{2.0925} \sqrt{\frac{1}{1} + \frac{1}{5} + \frac{(2.65 - 12.51/5)^2}{(2.0925)^2 \times 1.145}} = 0.076 \text{ mole }\%
\]

(b) For the mean of four measurements,

\[ s_c = \frac{0.1442}{2.0925} \sqrt{\frac{1}{4} + \frac{1}{5} + \frac{(2.65 - 12.51/5)^2}{(2.0925)^2 \times 1.145}} = 0.046 \text{ mole }\%\]
Interpretation of Least-Squares Results. The closer the data points are to the line predicted by a least-squares analysis, the smaller are the residuals. The sum of the squares of the residuals, $SS_{\text{resid}}$, measures the variation in the observed values of the dependent variable ($y$ values) that are not explained by the presumed linear relationship between $x$ and $y$.

$$SS_{\text{resid}} = \sum_{i=1}^{N} [y_i - (b + mx_i)]^2 \quad (8-19)$$

We can also define a total sum of the squares $SS_{\text{tot}}$ as

$$SS_{\text{tot}} = S_{yy} = \sum (y_i - \bar{y})^2 = \sum y_i^2 - \frac{(\sum y_i)^2}{N} \quad (8-20)$$

The total sum of the squares is a measure of the total variation in the observed values of $y$ since the deviations are measured from the mean value of $y$.

An important quantity called the **coefficient of determination** ($R^2$) measures the fraction of the observed variation in $y$ that is explained by the linear relationship and is given by

$$R^2 = 1 - \frac{SS_{\text{resid}}}{SS_{\text{tot}}} \quad (8-21)$$
The closer $R^2$ is to unity, the better the linear model explains the $y$ variations, as shown in Example 8-6. The difference between $SS_{tot}$ and $SS_{resid}$ is the sum of the squares due to regression, $SS_{regr}$. In contrast to $SS_{resid}$, $SS_{regr}$ is a measure of the explained variation. We can write

$$SS_{regr} = SS_{tot} - SS_{resid} \quad \text{and} \quad R^2 = \frac{SS_{regr}}{SS_{tot}}$$

By dividing the sum of squares by the appropriate number of degrees of freedom, we can obtain the mean square values for regression and for the residuals (error) and then the $F$ value. The $F$ value gives us an indication of the significance of the regression. The $F$ value is used to test the null hypothesis that the total variance in $y$ is equal to the variance due to error. A value of $F$ smaller than the value from the tables at the chosen confidence level indicates that the null hypothesis should be accepted and that the regression is not significant. A large value of $F$ indicates that the null hypothesis should be rejected and that the regression is significant.
Correlation coefficient

Pearson correlation coefficient:

\[ r = \Sigma[(x_i - \bar{x})(y_i - \bar{y}) / ns_x s_y] \]
\[ = [\Sigma x_i y_i - (nxy)] / [(\Sigma x_i^2 - nx^2)(\Sigma y_i^2 - ny^2)]^{1/2} \]
\[ = (n\Sigma x_i y_i - \Sigma x_i \Sigma y_i) / [\{n\Sigma x_i^2 - (\Sigma x_i)^2\}\{n\Sigma y_i^2 - (\Sigma y_i)^2\}]^{1/2} \]

\[ r_{\text{max}} = 1, \quad r_{\text{min}} = -1, \quad r = 0 \text{ (when } xy=0), \]

\[ 0.90 < r < 0.95 \quad \text{linearity} \]
\[ 0.95 < r < 0.99 \quad \text{good linearity} \]
\[ 0.99 < r \quad \text{excellent linearity} \]
EXAMPLE 8-6

Find the coefficient of determination for the chromatographic data of Example 8-4.

Solution

For each value of $x_i$, we can find a predicted value of $y_i$ from the linear relationship. Let us call the predicted values of $y_i$, $\hat{y}_i$. We can write $\hat{y}_i = b + mx_i$ and make a table of the observed $y_i$ values, the predicted values $\hat{y}_i$, the residuals $y_i - \hat{y}_i$, and the squares of the residuals $(y_i - \hat{y}_i)^2$. By summing the latter values, we obtain $SS_{\text{resid}}$ as shown in Table 8-2.
From Example 8-4, the value of $S_{yy} = 5.07748$. Hence,

$$R^2 = 1 - \frac{SS_{\text{resid}}}{SS_{\text{tot}}} = 1 - \frac{0.0624}{5.07748} = 0.9877$$

This calculation shows that over 98% of the variation in peak area can be explained by the linear model.

We can also calculate $SS_{\text{regr}}$ as

$$SS_{\text{regr}} = SS_{\text{tot}} - SS_{\text{resid}} = 5.07748 - 0.06240 = 5.01508$$

Let us now calculate the $F$ value. There were five $xy$ pairs used for the analysis. The total sum of the squares has 4 degrees of freedom associated with it since one is lost in
calculating the mean of the \( y \) values. The sum of the squares due to the residuals has 3 degrees of freedom because two parameters \( m \) and \( b \) are estimated. Hence \( SS_{\text{regr}} \) has only 1 degree of freedom since it is the difference between \( SS_{\text{tot}} \) and \( SS_{\text{resid}} \). In our case, we can find \( F \) from

\[
F = \frac{SS_{\text{regr}}/1}{SS_{\text{resid}}/3} = \frac{5.01508/1}{0.0624/3} = 241.11
\]

This very large value of \( F \) has a very small chance of occurring by random chance, and therefore, we conclude that this is a significant regression.
Transformed Variables. Sometimes an alternative to a simple linear model is suggested by a theoretical relationship or by examining residuals from a linear regression. In some cases linear least-squares analysis can be used after one of the simple transformations shown in Table 8-3.
Errors in External Standard Calibration

When external standards are used, it is assumed that, when the same analyte concentration is present in the sample and in the standard, the same response will be obtained. Thus, the calibration functional relationship between the response and the analyte concentration must apply to the sample as well.

Usually in a determination, the raw response from the instrument is not used. Instead, the raw analytical response is corrected by measuring a blank (see Section 5B-4). The ideal blank is identical to the sample but without the analyte. In practice, with complex samples, it is too time consuming or impossible to prepare an ideal blank, and so a compromise must be made. Most often a real blank is either a solvent blank, containing the same solvent in which the sample is dissolved, or a reagent blank, containing the solvent plus all the reagents used in sample preparation.

To avoid systematic errors in calibration, standards must be accurately prepared, and their chemical state must be identical to that of the analyte in the sample. The standards should be stable in concentration, at least during the calibration process. The accuracy of a determination can sometimes be checked by analyzing real samples of a similar matrix but with known analyte concentrations. Random errors can also influence the accuracy of results obtained from calibration curves.
Figure 8-11 Effect of calibration curve uncertainty. The dashed lines show confidence limits for concentrations determined by the regression line. Note that uncertainties increase at the extremities of the plot. Usually we estimate the uncertainty in analyte concentration only from the standard deviation of the response. Calibration curve uncertainty can significantly increase the uncertainty in the analyte concentration from \( sc \) to \( sc \ r \) as shown.
Multivariate Calibration

The least-squares procedure just described is an example of a univariate calibration procedure because only one response is used per sample. The process of relating multiple instrument responses to an analyte or a mixture of analytes is known as **multivariate calibration**. Multivariate calibration methods\(^2\) have become quite popular in recent years as new instruments are now available that produce multidimensional responses (absorbance of several samples at multiple wavelengths, mass spectrum of chromatographically separated components, and so on). Multivariate calibration methods are very powerful. They can be used to determine multiple components in mixtures simultaneously and can provide redundancy in measurements to improve precision. Recall that repeating a measurement \(N\) times provides a \(\sqrt{N}\) improvement in the precision of the mean value. These methods can also be used to detect the presence of interferences that would not be identified in a univariate calibration.

Multivariate techniques are **inverse calibration methods**. In normal least-squares methods, often called **classical least-squares methods**, the system response is modeled as a function of analyte concentration. In inverse methods, the concentrations are treated as functions of the responses. This latter approach can lead to some advantages in that concentrations can be accurately predicted even in the presence of chemical and physical sources of interference. In classical methods, all components in the system must be considered in the mathematical model produced (regression equation).
The common multivariate calibration methods are **multiple linear regression**, **partial least-squares regression**, and **principal components regression**.

These differ in the details of the ways in which variations in the data (responses) are used to predict the concentration. Software for accomplishing multivariate calibration is available from several companies.

The use of multivariate statistical methods for quantitative analysis is part of the subdiscipline of chemistry called **chemometrics**.
Minimizing Errors in Analytical Procedures

Separation
Sample cleanup by separation methods is an important way to minimize errors from possible interferences in the sample matrix.

Saturation, Matrix Modification, and Masking

Dilution and Matrix Matching

Internal Standard Methods
An internal standard is a reference species, chemically and physically similar to the analyte, that is added to samples, standards, and blanks. The ratio of the response of the analyte to that of the internal standard is plotted versus the concentration of analyte.

Standard addition methods
A known amount of a standard solution of analyte is added to one portion of the sample. The responses before and after the addition are measured and used to obtain the analyte concentration. Alternatively multiple additions are made to several portions of the sample. The standard additions method assumes a linear response.
Figure 8-12 Illustration of the internal standard method. A fixed amount of the internal standard species is added to all samples, standards, and blanks. The calibration curve plots the ratio of the analyte signal to the internal standard signal against the concentration of the analyte.
EXAMPLE 8-7

The intensities of flame emission lines can be influenced by a variety of instrumental factors, including flame temperature, flow rate of solution, and nebulizer efficiency. We can compensate for variations in these factors by using the internal standard method. Thus, we add the same amount of internal standard to mixtures containing known amounts of the analyte and to the samples of unknown analyte concentration. We then take the ratio of the intensity of the analyte line to that of the internal standard. The internal standard should be absent in the sample to be analyzed.

In the flame emission determination of sodium, lithium is often added as an internal standard. The following emission intensity data were obtained for solutions containing Na and 1000 ppm Li.
A plot of the Na emission intensity versus the Na concentration is shown in Figure 8-13a. Note that there is some scatter in the data and the $R^2$ value is 0.9816. In Figure 8-13b, the ratio of the Na to Li emission intensities is against the Na concentration. Note that the linearity is improved as indicated by the $R^2$ value of 0.9999. The unknown intensity ratio (0.0463) is then located on the curve, and the concentration of Na corresponding to this ratio is found to be $3.55 \pm 0.05$ ppm.

Figure 8-13 In (a) the Na flame emission intensity is plotted versus the Na concentration in ppm. The internal standard calibration curve is shown in (b), where the ratio of the Na to Li intensities is plotted versus the Na concentration.
EXAMPLE 8-8

The single-point standard addition method was used in the determination of phosphate by the molybdenum blue method. A 2.00-mL urine sample was treated with molybdenum blue reagents to produce a species absorbing at 820 nm, after which the sample was diluted to 100.00 mL. A 25.00-mL aliquot gave an instrument reading (absorbance) of 0.428 (solution 1). Addition of 1.00 mL of a solution containing 0.0500 mg of phosphate to a second 25.0-mL aliquot gave an absorbance of 0.517 (solution 2). Use these data to calculate the concentration of phosphate in milligrams per mL of the sample. Assume that there is a linear relationship between absorbance and concentration and that a blank measurement has been made.

Solution

The absorbance of the first solution is given by

\[ A_1 = kc_u \]

where \( c_u \) is the unknown concentration of phosphate in the first solution and \( k \) is a proportionality constant. The absorbance of the second solution is given by

\[ A_2 = \frac{kV_u c_u}{V_t} + \frac{kV_s c_s}{V_t} \]
where $V_u$ is the volume of the solution of unknown phosphate concentration (25.00 mL), $V_s$ is the volume of the standard solution of phosphate added (1.00 mL), $V_t$ is the total volume after the addition (26.00 mL), and $c_s$ is the concentration of the standard solution (0.500 mg mL$^{-1}$). If we solve the first equation for $k$, substitute the result into the second equation, and solve for $c_u$, we obtain

$$c_u = \frac{A_1 c_s V_s}{A_2 V_t - A_1 V_u} = \frac{0.428 \times 0.0500 \text{ mg mL}^{-1} \times 1.00 \text{ mL}}{0.517 \times 26.00 \text{ mL} - 0.428 \times 25.00 \text{ mL}} = 0.0780 \text{ mg mL}^{-1}$$

This is the concentration of the diluted sample. To obtain the concentration of the original urine sample, we need to multiply by 100.00/2.00. Thus,

$$\text{concentration of phosphate} = 0.00780 \text{ mg mL}^{-1} \times 100.00 \text{ mL}/2.00 \text{ mL} = 0.390 \text{ mg mL}^{-1}$$
8E Figures of Merit for Analytical Methods 
validation and reporting of analytical results

Analytical procedures are characterized by a number of figures of merit such as accuracy, precision, sensitivity, detection limit, and dynamic range.

The quality of the analytical method is decided by the following by the 6 “M”s:
Validation of analytical procedures is the process of determining the suitability of a given methodology for providing useful analytical data.

*J. Guerra, Pharm. Tech. March 1986*

Validation is the formal and systematic proof that a method compiles with the requirements for testing a product when observing a defined procedures.

*G. Maldener, Chromatographia, July 1989*

**USP 36 / Validation**

*Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications.*

Source: USP36,<1225> (2013).
http://www.drugfuture.com/Pharmacopoeia/USP32/pub/data/v32270/usp32nf27s0_c1058.html
Validation
(ISO 17025, 5.4.5.3 note 3)

Validation is always a balance between

Costs, Risks and Technical possibilities.

There are many cases in which the range and uncertainty of the values can only be given in a simplified way due to lack of information.

Dr. Manuela Schulze
Typical parameters used in analytical method validation
(USP 36 / ICH: International Conference on Harmonization)

- Accuracy
- Precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range
- Robustness / Ruggedness
- System suitability
8E-1 Sensitivity and Detection Limit

The definition of sensitivity most often used is the calibration sensitivity, or the change in the response signal per unit change in analyte concentration. The calibration sensitivity is thus the slope of the calibration curve, as shown in Figure 8-14.

Figure 8-14 Calibration curve of response $R$ versus concentration $c$. The slope of the calibration curve is called the calibration sensitivity $m$. The detection limit, DL, designates the lowest concentration that can be measured at a specified confidence level.
The **detection limit**, DL, is the smallest concentration that can be reported with a certain level of confidence. Every analytical technique has a detection limit. For methods that require a calibration curve, the detection limit is defined in a practical sense by Equation 8-22. It is the analyte concentration that produces a response equal to \( k \) times the standard deviation of the blank \( s_b \):

\[
DL = \frac{ks_b}{m}
\]  

(8-22)

where \( k \) is called the confidence factor and \( m \) is the calibration sensitivity. The factor \( k \) is usually chosen to be 2 or 3. A \( k \) value of 2 corresponds to a confidence level of 92.1%, while a \( k \) value of 3 corresponds to a 98.3% confidence level.\(^{24}\)

Detection limits reported by researchers or instrument companies may not apply to real samples. The values reported are usually measured on ideal standards with optimized instruments. These limits are useful, however, in comparing methods or instruments.
IUPAC defines LOD as follows:

The limit of detection, expressed as the concentration, $C_L$, or the quantity, $q_L$, is derived from the smallest measure, $x_L$, that can be detected with reasonable certainty for a given analytical procedure.

The value of $x_L$ is given by the equation

$$x_L = x_{bi} + ks_{bi}$$

where $x_{bi}$ is the mean of the blank measures, $s_{bi}$ is the standard deviation of the blank measures, and $k$ is a numerical factor chosen according to the confidence level desired.

The LOD definition by the ACS:

“the lowest concentration level that can be determined to be statistically different from a blank”

and is operationally defined as three times the standard deviation of measurements around the blank.

The American Chemical Society (ACS) committee on environmental improvement states LOD as the lowest concentration of an analyte that the analytical process can reliably detect.

- Chang KH. Limit of detection and its establishment in analytical chemistry. Health Env. J. 2011; 2(1) 38-43

\textbf{The minimum concentration of an analyte that can be identified, measured and reported with 99 \% confidence that the analyte concentration is greater than zero.}

European Pharmacopoeia definition based on Signal-to-noise ratio (S/N)

The S/N influences the quantification and is calculated from the equation:

\[
S/N = \frac{2H}{h}
\]

\(H\) = height of the peak corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half-height.

\(h\) = range of the background noise in a chromatogram obtained after injection or application of a blank, observed over a distance equal to 20 times the width at half-height of the peak in the chromatogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

Source: *European Pharmacopoeia 5.0  2.2.46.*
Approaches for the estimation of LOD and LOQ

1. **Calibration line method** (ISO definition)

According to ISO 11843-2 or the German standard DIN 32645

From the **residual standard deviation** of the calibration line
Calculation from the 95% estimation interval of the calibration line

\[
\text{LOD} = \text{intercept} + (3 \times \text{SD}_y / \text{slope}) = b + (3 \times \text{SD}_y / m)
\]

- Internal standard calibration
- External standard calibration

Models
- Linear regression: Ordinary least square
- Weighted least square regression
- Non-linear second order calibration function
  \[y = a + bx + cx^2\]
\[ y(\pm SD_y) = m(\pm SD_m) x + b(\pm SD_b) \]
Definition: A predicted value (or fitted value) is the predicted value of \( y_i \) for a given \( x_i \) based on the regression equation \( b_0 + b_1 x_i \).

\( b_1 \) is the slope.

Notation: \( \hat{y}_i = b_0 + b_1 x_i \)

A residual is the departure from \( Y \) of a fitted value

Notation: \( \text{resid}_i = y_i - \hat{y}_i \)

The residual sum of squares (a.k.a. SS(resid) or SSE) is

\[
SS(\text{resid}) = SSE = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2
\]

\[
= \sum_{i=1}^{n} (y_i - (b_0 + b_1 x_i))^2
\]

Using calculus to minimize the SSE, we find the coefficients for the regression equation.

\[
b_1 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{n} (x_i - \bar{x})^2} \quad b_0 = \bar{y} - b_1 \bar{x}
\]
Residual standard deviation:

the standard deviation of the residuals (residuals = differences between observed and predicted values). It is calculated as follows:

\[ SD_{\text{res}} = \sqrt{\frac{\sum(y - y_{\text{est}})^2}{n - 2}} \]
2. Calculation from SD of the Blank (IUPAC definition)

\[
\text{LOD} = \text{mean}_{\text{blank}} + 3 \times S_{\text{blank}}
\]

German standard  DIN 32645

DIN 32645

\[
\begin{align*}
\text{LOD} &= 6 \times S_{\text{blank}} / b & \text{LOQ} &= 9 \times S_{\text{blank}} / b \\
n &= 10, \quad \alpha = \beta = 0.01 \\
\text{Regulation 333/2007} & \\
\text{LOD} &= 3 \times S_{\text{blank}} & \text{LOQ} &= 6 \times S_{\text{blank}} \\
n &= 20, \quad \alpha = \beta = 0.05
\end{align*}
\]

\( S_{\text{blank}} \) = standard deviation of the measurements at the blank level

\( b \) = slope of the calibration curve

Software: Chemstation, LIMS, DINTEST, Valistat, Effichem, MVA3 etc.
Recently, the ISO recommended a definition of the minimum limit of detection in relation to stated levels of Type I and II errors. The default levels for these errors were set to 5%, i.e., $\alpha = \beta = 5\%$, $p_\alpha = p_\beta = 0.05$. An value of 5% corresponds to using the 95% of the distribution of blank values as the limit for declaring a measured value significantly higher than the blank. Given a gaussian distribution of blank values, this limit corresponds to:

$$\text{LoB} = \text{mean}_{\text{blank}} + 1.645(\text{SD}_{\text{blank}})$$

$$\text{LoD} = \text{LoB} + 1.645(\text{SD}_{\text{low concentration sample}})$$
3. Visual evaluation

LOD is determined by the analysis of a series of samples with known concentrations and establishing the minimum level at which the analyte can be reliably detected.

4. S/N ratio (European Pharmacopeia definition)

\[ S/N = \frac{2H}{h} \]

\[
\begin{align*}
\text{LOD} & = 3 \times S/N \\
\text{LOQ} & = 10 \times S/N
\end{align*}
\]

The measurement for S/N (signal to noise) is not an exact science. Meaning that there are many different ways to make the determination and some of those ways are a bit arbitrary as the value of the noise is made by an "eyeball" estimate.
Graph of a sine wave's voltage vs. time (in degrees), showing RMS, peak, and peak-to-peak voltages

**PEAK TO PEAK VALUE:**
The peak 2 peak value of a sine wave is the value of voltage (or current) from the positive peak to negative peak.

\[ V_{pp} = 2V_p \quad I_{pp} = 2I_p \]

**RMS VALUE:**
The term rms stand for root mean square. It is also referred to as the effective value.

\[ V_{rms} = 0.707 \, V_p \quad I_{rms} = 0.707 \, I_p \]

6 Standard deviation ---- 99.7%
6.6 Standard deviation ---- 99.9%
8E-2 Linear Dynamic range

The **linear dynamic range** of an analytical method most often refers to the concentration range over which the analyte can be determined using a linear calibration curve (see Figure 8-14).

The **lower limit** of the dynamic range is generally considered to be the detection limit.

The **upper end** is usually taken as the concentration at which the analytical signal or the slope of the calibration curve. Usually a deviation of 5% from linearity is considered the upper limit. Deviations from linearity are common at high concentrations because of nonideal detector responses or chemical effects. Some analytical techniques, such as absorption spectrophotometry, are linear over only **one to two orders** of magnitude. Other methods, such as mass spectrometry, may exhibit linearity over **four to five orders** of magnitude. In some determinations, only a small dynamic range is required. For example, in the determination of sodium in blood serum, only a small range is needed because variations of the sodium level in humans is quite limited.
Quality Assurance of Analytical Results

Control Charts
A control chart is a sequential plot of some quality characteristic that is important in quality assurance. The chart also shows the statistical limits of variation that are permissible for the characteristic being measured.

We can then determine whether the measurements on consecutive days are within certain limits of the standard mass. These limits are called the upper and lower control limits (UCL and LCL), defined as

\[
UCL = \mu + \frac{3\sigma}{\sqrt{N}} \\
LCL = \mu - \frac{3\sigma}{\sqrt{N}}
\]

where \(\mu\) is the population mean for the mass measurement, \(\sigma\) is the population standard deviation for the measurement, and \(N\) is the number of replicates that are obtained for each sample. The population mean and standard deviation for the standard mass must be estimated from preliminary studies. Note that the UCL and the LCL are three standard deviations on either side of the population mean and form a range within which a measured mass is expected to lie 99.7% of the time.
Figure 8–15 A control chart for a modern analytical balance. The results appear to fluctuate normally about the mean except for those obtained on day 17. Investigation led to the conclusion that the questionable value resulted from a dirty balance pan. UCL 5 upper control limit; LCL 5 lower control limit.
Figure 8-15 is a typical instrument control chart for an analytical balance. Mass data were collected on twenty-four consecutive days for a 20.000-g standard mass certified by the National Institute of Standards and Technology. On each day, five replicate determinations were made. From independent experiments, estimates of the population mean and standard deviation were found to be \( \mu = 20.000 \text{ g} \) and \( \sigma = 0.00012 \text{ g} \), respectively. For the mean of five measurements, \( 3 \times \left( \frac{0.00012 \text{ g}}{\sqrt{5}} \right) = 0.00016 \). Hence, the UCL value = 20.00016 g, and the LCL value = 19.99984 g. With these values and the mean masses for each day, the control chart shown in Figure 8-15 can be constructed. As long as the mean mass remains between the LCL and the UCL, the balance is said to be in **statistical control**. On day 17, the balance went out of control, and an investigation was launched to find the cause for this condition. In this example, the balance was not properly cleaned on day 17 so that there was dust on the balance pan. Systematic deviations from the mean are relatively easy to spot on a control chart.
In another example, a control chart was used to monitor the production of medications containing benzoyl peroxide used for treating acne. Benzoyl peroxide is a bactericide that is effective when applied to the skin as a cream or gel containing 10% of the active ingredient. These substances are regulated by the Food and Drug Administration (FDA). Concentrations of benzoyl peroxide must, therefore, be monitored and maintained in statistical control. Benzoyl peroxide is an oxidizing agent that can be combined with an excess of iodide to produce iodine that is titrated with standard sodium thiosulfate to provide a measure of the benzoyl peroxide in the sample.

The control chart of Figure 8-16 shows the results of 89 production runs of a cream containing a nominal 10% benzoyl peroxide measured on consecutive days. Each sample is represented by the mean percent benzoyl peroxide determined from the results of five titrations of different analytical samples of the cream.

The chart shows that, until day 83, the manufacturing process was in statistical control with normal random fluctuations in the amount of benzoyl peroxide. On day 83, the system went out of control with a dramatic systematic increase above the UCL. This increase caused considerable concern at the manufacturing facility.
until its source was discovered and corrected. These examples show how control charts are effective for presenting quality control data in a variety of situations.

**Figure 8-16** A control chart for monitoring the concentration of benzoyl peroxide in a commercial acne preparation. The manufacturing process became out of statistical control with sample 83 and exhibited a systematic change in the mean concentration.
Validation

Validation determines the suitability of an analysis for providing the sought-for information and can apply to samples, to methodologies, and to data. Validation is often done by the analyst, but it can also be done by supervisory personnel.

Validation of samples is often used to accept samples as members of the population being studied, to admit samples for measurement, to establish the authenticity of samples, and to allow for resampling if necessary. In the validation process, samples can be rejected because of questions about the sample identity, questions about sample handling, or knowledge that the method of sample collection was not appropriate or in doubt. For example, contamination of blood samples during collection as evidence in a forensic examination would be reason to reject the samples.

There are several different ways to validate analytical methods. Some of these were discussed in Section 5B-4. The most common methods include analysis of standard reference materials when available, analysis by a different analytical method, analysis of “spiked” samples, and analysis of synthetic samples approximating the chemical composition of the test samples. Individual analysts and laboratories often must periodically demonstrate the validity of the methods and techniques used.

Data validation is the final step before release of the results. This process starts with validating the samples and methods used. Then, the data are reported with statistically valid limits of uncertainty after a thorough check has been made to eliminate blunders in sampling and sample handling, mistakes in performing the analysis, errors in identifying samples, and mistakes in the calculations used.
**Reporting Analytical Results**

Specific reporting formats and procedures vary from laboratory to laboratory. However, a few general guidelines can be mentioned here. Whenever appropriate, reports should follow the procedure of a good laboratory practice (GLP). Generally, **analytical results should be reported as the mean value and the standard deviation.** Sometimes the standard deviation of the mean is reported instead of that of the data set. Either of these is acceptable as long as it is clear what is being reported. A **confidence interval** for the mean should also be reported. Usually the 95% confidence level is a reasonable compromise between being too inclusive or too restrictive.

Again, the interval and its confidence level should be explicitly reported. The results of various statistical tests on the data should also be reported when appropriate, as should the rejection of any outlying results along with the rejection criterion. Significant figures are quite important when reporting results and should be based on statistical evaluation of the data. Whenever possible the significant figure convention stated in Section 6D-1 should be followed. Rounding of the data should be done with careful attention to the guidelines.

Whenever possible graphical presentation should include error bars on the data points to indicate uncertainty. Some graphical software allows the user to choose different error bar limits of $61s$, $62s$, and so forth, while other software packages automatically choose the size of the error bars. Whenever appropriate the regression equation and its statistics should also be reported.
Summary

Sampling standardsization calibration
Matrix effect quartering gross sample
Bulk sample homogeneous random sampling
Lab sample Tedlar® bag throughput
Lake stratification Linear regression residual
Least Squares Method calibration curve correlation coefficient
external standards blank internal standard
Standard addition multivariate statistical methods
Chemometrics validation detection limit (DL)
LOD dynamic range Quality assurance
control charts GLP