Advanced Techniques For Pharmaceutical Analysis

المحاضرة رقم (1)

Quality Assurance ضمان الجودة

The analytical approach, appears to be a straightforward process of moving from problem-to-solution in five-step process:

(1) Identify and define the problem;
(2) Design the experimental procedure;
(3) Conduct an experiment and gather data;
(4) Analyze the experimental data; and
(5) Propose a solution to the problem.

The "feedback loop" in the analytical approach is maintained by a quality assurance program (Figure 1).

A quality assurance program identifies the practices necessary to :

***bring a system into statistical control,**
***allows us to determine if the system remains in statistical control, and**
***suggests a course of corrective action when the system has fallen out of statistical control.**

Quality assurance **The steps taken during an analysis to ensure that the analysis is under control and that it is properly monitored.**

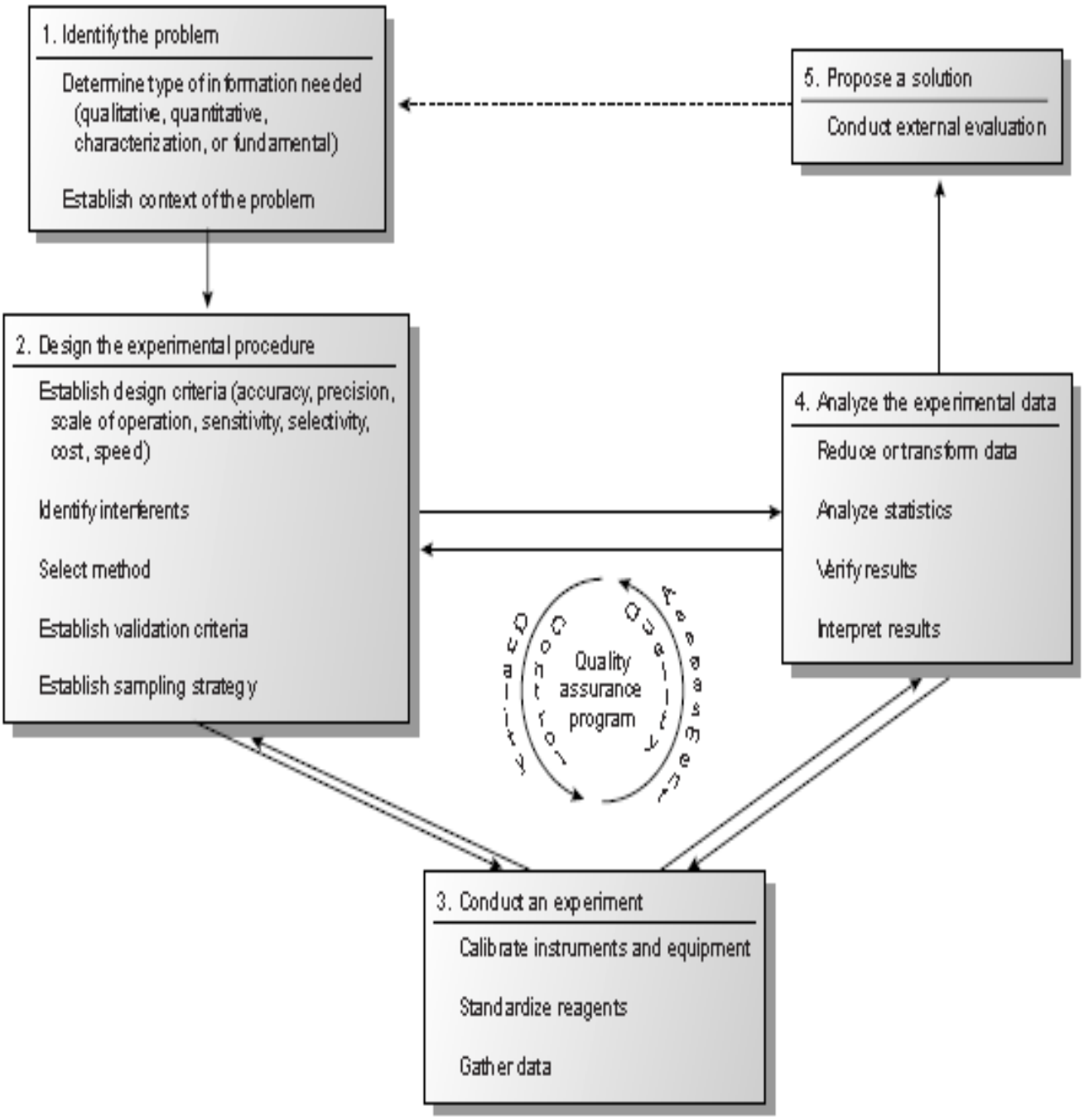

Figure 1: Schematic diagram of the analytical approach to problem solving, showing the role of the quality assurance program

The principal components of a quality assurance program:

1) **quality control**

2) **quality assessment.**

Quality Control

Quality control encompasses all activities used to bring a system into statistical control. The most important facet of quality control is a set of written directives describing all relevant:

1-laboratory-specific,

2-technique-specific,

3-sample-specific,

4-method-specific, and

5-protocol-specific operations.

Quality Assessment

The goals of quality assessment are:

***to determine when a system has reached a state of statistical control;**

***to detect when the system has moved out of statistical control; and,**

***to suggest why a loss of statistical control has occurred so that corrective actions can be taken.**

For convenience, the methods of quality assessment are divided into two categories:

1-internal methods that are coordinated within the laboratory and

2-external methods for which an outside agency or individual is responsible.

1-Internal Methods of Quality Assessment

Internal methods of quality assessment are:

1-the analysis of duplicate samples,

2-the analysis of blanks,

3-the analysis of standard samples, and

4-spike recoveries.

***Analysis of Duplicate Samples** **An effective method for determining the precision of an analysis is to analyze duplicate samples. The results from the duplicate samples, X_1 and X_2, are evaluated and comparing the results with accepted values.**

If duplicate samples from several sources are combined, then the precision of the measurement process must be approximately the same for each.

***The Analysis of Blanks** **The use of a blank as a means of correcting the measured signal for contributions from sources other than the analyte. The most common blank is a method, or reagent blank, in which an analyte-free sample, usually distilled water, is carried through the analysis using the same reagents, glassware, and instrumentation.**

***Analysis of Standards** **The analysis of a standard containing a known concentration of analyte also can be used to monitor a system's state of statistical control.**

***Spike Recoveries** **One of the most important quality assessment tools is the recovery of a known addition, or spike, of analyte to a method blank or sample. To determine a spike recovery, the blank or sample is split into two portions, and a known amount of a standard solution of the analyte is added to one portion.**

The concentration of the analyte is determined for both the spiked, F, and unspiked portions, I, and the percent recovery, %R, is calculated as

$$\%R = [(F - I) / A] \times 100$$

where A is the concentration of the analyte added to the spiked portion.

spike recovery

An analysis of a sample after spiking with a known amount of analyte.

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A spike recovery for the analysis of chloride in well water was performed by adding 5.00 mL of a 25,000-ppm solution of Cl^- to a 500-mL volumetric flask and diluting to volume with the sample. Analysis of the sample and the spiked sample resulted in chloride concentrations of 183 ppm and 409 ppm, respectively. Determine the percent recovery of the spike.

SOLUTION

The concentration of the added spike is calculated by taking into account the effect of dilution.

$$A = 25,000 \text{ ppm} \times \frac{5.00 \text{ mL}}{500.0 \text{ mL}} = 250 \text{ ppm}$$

Thus, the spike recovery is

$$\%R = \frac{409 - 183}{250} \times 100 = 90.4\%$$

<u>Spike recoveries for samples are used to detect systematic errors due to the sample matrix or the stability of the sample after its collection</u>.

2-External Methods of Quality Assessment

One external method of quality assessment is the certification of a laboratory by a sponsoring agency. Certification is based on the successful analysis of a set of proficiency standards prepared by the sponsoring agency.

Sample Preparation

***The measurement process**

***Errors in quantitative analysis**

*** Method performance and method validation**

*** Preservation of samples**

I]-The measurement process

Some common steps involved in the process are shown in Figure 1.

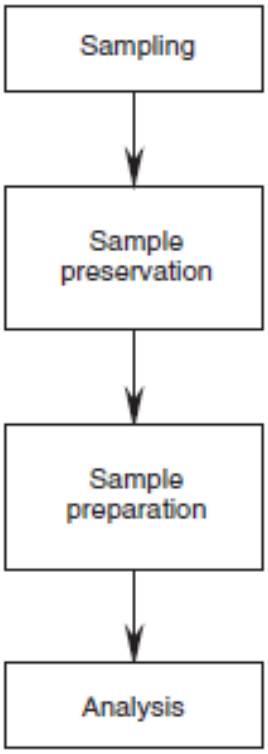

Figure 1: Steps in a measurement process.

The first step is sampling , where the sample is obtained from the object to be analyzed. 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.

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.

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.

II]- 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. It can also be reduced with improved techniques. In general, errors can be classified as random and systematic.

Accuracy

Accuracy is the closeness of an experimental measurement or result to the true value.

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:

$$\text{accuracy} = (\text{mean} - \text{true value}) / \text{true value}$$

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.

Precision

Precision is the closeness of agreement between replicated measurements or results obtained under the same prescribed conditions.
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.

In general, the smaller the spread of values or deviations, the smaller the value of σ and hence the better the precision.

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, $\bar{x}$:

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

Σx_i is the sum of the replicate measurements and n is the total number of measurements. The common measure of variability (or precision) is the standard deviation, σ.

This is calculated as

$$\sigma = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

Another term commonly used to measure variability is the relative standard deviation (RSD), which may also be expressed as a percentage:

$$\text{RSD} = \sigma / \bar{x} \qquad \text{or} \qquad \%\ \text{RSD} = \sigma / \bar{x} \times 100$$

Relative standard deviation is the parameter for expressing precision in analytical sciences.

To reducing uncertainty during sample preparation are given by:

Minimize the Number of Steps

When multiple steps such as those shown in Figure 5 are involved, the uncertainty is compounded. A simple dilution example presented in Figure 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. The greater the number of steps, the more errors there are.

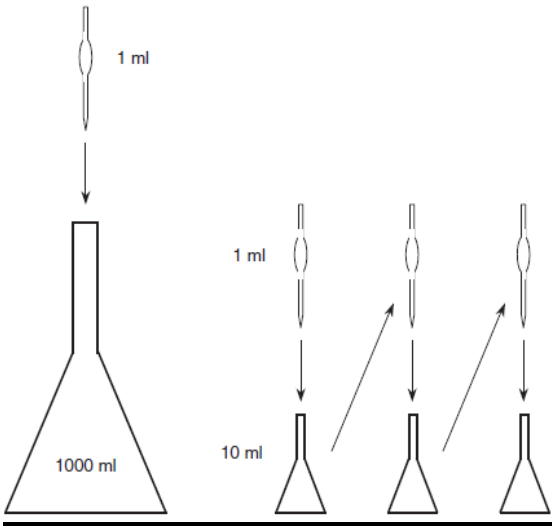

Figure 5. Examples of single and multiple dilution of a sample.

III]- Method performance and method validation

The criteria used for evaluating analytical methods are called figures of merit. The figures of merit are listed in Table 2. Accuracy and precision have already been discussed; other important characteristics are sensitivity, detection limits, and the range of quantitation.

Table 2. Figures of Merit for Instruments or Analytical Methods

No.	Parameter	Definition
1	Accuracy	Deviation from true value
2	Precision	Reproducubility of replicate measurements
3	Sensitivity	Ability to discriminate between small differences in concentration
4	Detection limit	Lowest measurable concentration
5	Linear dynamic range	Linear range of the calibration curve
6	Selectivity	Ability to distinguish the analyte from interferances
7	Speed of analysis	Time needed for sample preparation and analysis
8	Ease of automation	How well the system can be automated
9	Ruggedness	Durability of measurement, ability to handle adverse conditions
10	Robustness	Examined by evaluating the effect of small change in some of the most important procedure parameters
11	Cost	labor Equipment cost + cost of supplies

Sensitivity

The sensitivity of a method (or an instrument) is a measure of its ability to distingueish 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

$$S = mc + s_{bl} \qquad (1)$$

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.

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. Therefore, the minimum detectable concentration C_m is:

$$C_m = (s_m - s_{bl}) / m \qquad (2)$$

where s_m is the smallest distinguishable signal.

IV]- 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.

The sample collected is exposed to conditions different from the original source. For example, analytes in a ground water sample that have never been exposed to light can undergo significant photochemical reactions when exposed to sunlight. 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.

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

Choice of Proper Containers

The surface of the sample container may interact with the analyte. For metals can adsorb irreversibly on glass surfaces, so plastic containers are chosen for holding water samples to be analyzed for their metal content. Organic molecules may also interact with polymeric container materials. Consequently, glass containers are suitable for organic analytes.