

Analytical Procedures

The stages or steps in an overall analytical procedure can be summarised as follows:

Definition of the problem

Analytical information and level of accuracy required. Costs, timing, availability of laboratory instruments and facilities.

Choice of technique and method

Selection of the best technique for the required analysis, such as chromatography, infrared spectrometry, titrimetry, thermogravimetry. Selection of the method (i.e. the detailed stepwise instructions using the selected technique).

Sampling

Selection of a small sample of the material to be analysed. Where this is heterogeneous, special procedures need to be used to ensure that a genuinely representative sample is obtained.

Sample pre-treatment or conditioning

Conversion of the sample into a form suitable for detecting or measuring the level of the analyte(s) by the selected technique and method. This may involve dissolving it, converting the analyte(s) into a specific chemical form or separating the analyte(s) from other components of the sample (the sample matrix) that could interfere with detection or quantitative measurements.

Qualitative analysis

Tests on the sample under specified and controlled conditions. Tests on reference materials for comparison. Interpretation of the tests.

Quantitative analysis

Preparation of standards containing known amounts of the analyte(s) or of pure reagents to be reacted with the analyte(s). Calibration of instruments to determine the responses to the standards under controlled conditions. Measurement of the instrumental response for each sample under the same conditions as for the standards. All measurements may be replicated to improve the reliability of the data, but this has cost and time implications. Calculation of results and statistical evaluation.

Preparation of report or certificate of analysis

This should include a summary of the analytical procedure, the results and their statistical assessment and details of any problems encountered at any stage during the analysis.

Review of the original problem

The results need to be discussed with regard to their significance and their relevance in solving the original problem. Sometimes repeat analyses or new analyses may be undertaken.

INSTRUMENTAL ANALYSIS

Essentially all analytical instruments are electrically operated. An understanding of the operation of the electrical components of an instrument can aid in locating a malfunctioning portion of the instrument and can make it possible for the analyst to obtain maximal use and information from the instrument. In

changes occur that could affect the validity of the analytical data. The following effects during storage should be considered:

1. Increases in temperature leading to the loss of volatile analytes, thermal or biological degradation or increased chemical reactivity.
2. Decreases in temperature that lead to the formation of deposits or the precipitation of analytes with low solubilities.
3. Changes in humidity that affect the moisture content of hygroscopic solids and liquids or induce hydrolysis reactions.
4. UV radiation, particularly from direct sunlight, that induces photochemical reactions, photodecomposition or polymerisation.
5. Air-induced oxidation.
6. Physical separation of the sample into layers of different density or changes in crystallinity.

In addition, containers may leak or allow contaminants to enter.

A particular problem associated with samples having very low (trace and ultra-trace) levels of analytes in solution is the possibility of losses by adsorption onto the walls of the container or contamination by substances being leached from the container by the sample solvent. Trace metals may be depleted by adsorption or ion-exchange processes if stored in glass containers, whilst sodium, potassium, boron and silicates can be leached from the glass into the sample solution. Plastic containers should always be used for such samples.

Conversely, sample solutions containing organic solvents and other organic liquids should be stored in glass containers because the base plastic or additives such as plasticisers and antioxidants may be leached from the walls of plastic containers.

SAMPLE PRE-TREATMENT

Samples arriving in an analytical laboratory come in a very wide assortment of sizes, conditions and physical forms and can contain analytes from major constituents down to ultra-trace levels. They can have a variable moisture content and the matrix components of samples submitted for determinations of the same analyte(s) may also vary widely. A preliminary or pre-treatment, is often used to condition them in readiness for the application of a specific method of analysis or to pre-concentrate (enrich) analytes present at very low levels. Examples of pre-treatments are:

1. Drying at 100 to 120°C to eliminate the effect of a variable moisture content;
2. Weighing before and after drying enables the water content to be calculated or it can be established by thermogravimetric analysis.
3. Separating the analytes into groups with common characteristics by distillation, filtration, centrifugation, solvent or solid phase extraction.
4. Removing or reducing the level of matrix components that are known to cause interference with measurements of the analytes.
5. Concentrating the analytes if they are below the concentration range of the analytical method to be used by evaporation, distillation, co-precipitation, ion-exchange, solvent or solid phase extraction or electrolysis.

Sample clean-up in relation to matrix interference and to protect specialised analytical equipment such as chromatographic columns and detection systems from high levels of matrix components is widely practised using solid phase extraction (SPE) cartridges. Substances such as lipids, fats, proteins, pigments, polymeric and tarry substances are particularly detrimental.

$$\sigma = \sqrt{\frac{\sum (x_i - \mu)^2}{N}}$$

It should be kept in mind that the equation holds strictly only as $N \rightarrow \infty$. In actual practice we must calculate the individual deviations from the mean of limited number of measurements, \bar{x} , in which it is anticipated that $\bar{x} \rightarrow \mu$, although it may not be certain.

For a set of N measurements, it is possible to calculate N independently variable deviations from some reference number. It should be kept in mind that if the reference number chosen is the estimated mean, \bar{x} , the sum of the individual deviations must necessarily add up to zero and thus value of $N-1$ deviations are adequate to define the N th.

The estimated standard deviation, s , finite set of experimental data more nearly approximates σ if the number of degrees of freedom is substituted for N .

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N - 1}}$$

Confidence Limits

Calculation of the standard deviation from a set of data provides an indication of the precision inherent in a particular analysis. Now unless there are large number of data, it does not by itself give any information regarding the experimentally determined mean \bar{x} which might be to the true mean value μ . Statistical theory allows us to determine the range within which the true value might fall. This range is called the confidence interval and the limits of this range are called the confidence limit. The confidence limit is given by the relation:

$$\text{Confidence limit} = \bar{x} \pm \frac{ts}{\sqrt{N}}$$

where, t is statistical factor that depends on the number of degrees of freedom and the confidence limit described. The values of t for degrees of freedom for various confidence limits are given in Table 2.2. It should be noted that the confidence limit is simply the product of t and the standard deviation of the mean (s/\sqrt{N}).

Table 2.2. Values of t for ν degrees of freedom for various confidence levels.

| ν | Confidence levels (%) | | | |
|----------|-----------------------|--------|--------|--------|
| | 90 | 95 | 99 | 99.5 |
| 1. | 6.314 | 12.706 | 63.657 | 127.32 |
| 2. | 2.920 | 4.303 | 9.925 | 14.089 |
| 3. | 2.353 | 3.182 | 5.841 | 7.453 |
| 4. | 2.132 | 2.776 | 4.604 | 5.598 |
| 5. | 2.015 | 2.571 | 4.032 | 4.773 |
| 15. | 1.753 | 2.131 | 2.947 | 3.252 |
| 25. | 1.708 | 2.060 | 2.787 | 3.078 |
| ∞ | 1.645 | 1.960 | 2.576 | 2.807 |

concept noted above. To compare two different approaches to chromatographic data analysis, we could build up a collection of representative and problem chromatograms, feed them to both algorithms, and compare the results.

The comparison is much more useful than duplicate injections because small variations in actual sample handling can bring the reality of small differences in results into question. Using data files as input means that the data are exactly the same and any differences are significant.

Laboratory Information Management Systems (LIMSs)

LIMSs originally developed as sample tracking systems. Instrument data systems handled the laboratory data and the administrative information about samples was managed by the LIMS. Things have become a bit more complex since then.

There are three major viewpoints concerning the nature of LIMS:

1. LIMS is the centerpoint of all laboratory activities; it coordinates them and manages the interfaces and data collection.
2. LIMS is not a product, but rather a concept that encompasses the entire laboratory.
3. LIMS really means sample tracking and management and is only one component of a laboratory-wide structure.

These choices are not a matter of right or wrong, but of how you want your laboratory to work.