

Chemical Lab

Chemistry is the science of matter at the atomic to molecular scale, dealing primarily with collections of atoms (such as molecules, crystals, and metals). Chemistry deals with the composition and statistical properties of such structures, as well as their transformations and interactions often to become materials encountered in everyday life.

Chemistry also deals with understanding the properties and interactions of individual atoms with the purpose of integration of this knowledge with known facts about larger scale matter in order to obtain a useful insights or make accurate predictions According to modern chemistry, the physical properties of materials are generally determined by their structure at the atomic level which is, itself, determined by the properties and energies of the interactions between atoms and molecules.

Chemistry also involves testing which ranges from qualitative tests, designed to identify specific compounds or chemical groups, to quantitative testing which provides measurements of amounts of specific entities present or measurement of properties.

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1.Key Functions - Introductions

Chemical Lab deals with several types of products and tests. The product range includes and not limited to:

1. Testing of potable waters for properties such as: color, odor, taste, pH, total dissolved solids,, turbidity etc. Waters may also be tested for specific chemical determinands such as chloride, sulphate, carbonate & bi-carbonate, nitrate, sodium, calcium, manganese, iron etc. Organic contaminants are also often of interest such as volatile and semi-volatile materials including polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls(PCB) and pesticides.

2. Food and agricultural produce may undergo testing for proximate parameters such as moisture (water) content, ash, acid insoluble ash, fat, protein, carbohydrate, crude and dietary fibers and mineral content. Specific materials or groups of similar substances may also need to be measured. These include fatty acid profiling, vitamin profile, amino acid profile. Specific contaminant analysis may also be carried out to determine such things as levels of toxic heavy metals, micotoxins and residual pesticides. Other tests might include package integrity, tests on packaging materials for potential to contaminate food and shelf life evaluation.

3. Particular types of products may have their own appropriate testing regimes. edible oils are typically tested for color, refractive index, iodine value, peroxide value, saponification value, Unsaponifiable matter, adulterants, anti-oxidants fatty acid profile etc.

Pharmaceuticals and Drugs will be subject to tests to determine, identification especially of active principal, moisture, ash, purity, properties of preparation such as tablets, capsules, syrups and presence of specific impurities. There may also be tests specific to particular preparations and actives as delineated in pharmacopoeias.

Metals and alloys may be tested for chemical composition and physical properties to establish suitability for particular applications.

Ores and minerals may be tested for chemical composition and to establish their identity. In the case of ores the quantitative composition will be of particular interest so as to establish economic value.

Petroleum products and fuels will undergo tests such as calorific value, moisture content, ash content and relevant physical properties such as viscosity and volatility. Screening for damaging impurities will also be carried out

2.Design, Development and Layout

Design of Laboratory

Laboratories must provide a safe working environment which complies with current thinking on energy conservation and environmental impact of activities.

It must also be properly equipped with services such as a stable and reliable electricity supply, good ventilation with fume extraction where necessary, adequate lighting, safety systems such as fire control measures, secure and protected storage for records, including computer back-ups and water and gas supplies.

Laboratories must also be secure to provide for client confidentiality and so as to ensure that data and samples can only be accessed by authorised personnel. There must also be adequate arrangements to provide and to monitor any special conditions required for different types of testing. For example microbiology testing areas must be designed so as to be easily cleaned and areas for textile testing require close control, of temperature and humidity. Care must also be taken to ensure that incompatible activities are clearly separated, e.g. handling of high and low level samples, separation of areas for handling standards and samples. This will normally be achieved by a combination of management practices and physical segregation.

All health and safety hazard must be anticipated and carefully evaluated so that protective measures can be incorporated into the design. No matter, how well designed a laboratory is, improper usage of its facilities will defeat the engineered safety features. Proper education for the facility users is essential. The information given is applicable to all Laboratory buildings, laboratory units and laboratory work areas in which hazardous materials are used, handled or stored.

The design aspects are classified and discussed in detail as given

- 1 General requirements for laboratory.
- 2 Electrical safety.
- 3 Laboratory ventilation and Fume hoods.
- 4 Emergency eye wash and Safety shower.
- 5 Pressure vessel components and compressed gas cylinders.
- 6 Hazardous material storage cabinets.
- 7 Model Floor Plans.
- 8 Relevant Links.

1. General requirements for laboratory.

a. Building Requirements

i Designer Qualifications- The designer shall have appropriate professional licence in his/her area of expertise.

ii Building Occupancy Classification- Occupancy classification is to be based upon an assessment of a projected chemical inventory of the building. Prior to the final design, the campus fire safety organization will need to assign an occupancy class to insure compliance with the building codes.

iii Environmental Aspects- Identify pollution abatement engineering requirement for the building, well before resource allocation decisions are made

b. Building Design Issues

Handling and storage of hazardous materials inherently carries a higher risk of exposure and injury. It is important to segregate hazardous and non hazardous areas. Following aspects or to be considered while designing the laboratory:

Choice of fire proof construction for the building.

Separate office spaces for laboratory employees. It is prohibited to store and consume food, apply make-up or chew gum in areas where hazardous materials are used/stored.

Public access to laboratory personnel in office rooms with separate corridor access is highly desirable.

An automatically triggered main gas shut-off valve and interior shut-off valves shall be provided.

Large section of glass shall be shatter resistant.

c. Laboratory Design Considerations

It shall be completely separated from outside areas.

The design shall allow to accommodate additional facilities such as construction of workplace/storage area etc.

Mechanical climate control shall be provided.

The design shall allow adequate office space, isolated from the laboratory, but still near.

The air supply and exhaust vents shall be designed so as to minimize the potential for inhalation of chemicals.

Workstations in the laboratory need to accommodate computer monitors, keyboards and work holders and shall be height adjustable.

Each room in the hazardous area of the laboratory shall contain a sink with proper plumbing for hand washing.

All bench tops counters etc. shall be impervious to the chemicals and materials used in the laboratory.

The design shall allow easy cleaning (at the corners and floor areas beneath the work benches).

Flooring in chemicals use areas shall be chemical resistant and shall have covings to the wall.

The wall shall be non-porous and painted with a durable, impervious paint.

Effluent collection ports shall be provided.

Electrical insulation shall be provided.

Laboratory areas shall be well lit.

d. Hazardous Materials Design Issues.

Facilities shall be designed, so that use of a respirator is not required for normal operations.

A pressure differential system should be used to control the flow of airborne contamination.

The flow shall be from clean areas to contaminated areas

An area for storage of spill or emergency response equipment shall be located on each floor.

Sufficient space or facilities such as storage cabinets with partitions etc. shall be provided.

e. Entries, Exits etc...

The main emergency egress from the laboratory shall have a minimum clearance of 36 inches.

To prevent blocking egress, lab benches, and other furniture shall not be placed so that there is less than 5 feet of clear egress.

Space between benches, cabinets, and equipment shall be easily accessible for cleaning and servicing

Automatic self closing doors are advisable and shall be able to open with minimum effort.

All exit and emergency doors serving hazardous occupancies shall swing in the direction of exit travel.

f. Electrical and Utility issues

Laboratory shall be fitted with electrical outlets that can accommodate current requirements with an additional 40% capacity.

Circuit breakers shall be located outside the laboratory area.

Main valves for gas and vacuum lines shall be located outside the laboratory area.

Flexible connections shall be used for connecting gas and other plumbed utilities.

g. Accessibility

Laboratory design shall include adapted workbenches as necessary.

A work surface that can be adjusted to be from 27 to 37 inches from the floor must be provided.

A 29 inch clearance beneath the top to a depth of at least 20 inches.

A minimum width of 36 inches to allow for leg space for the seated individuals

2. Electrical safety.

Shall provide GFI protection to electrical connection points above counter tops and within 6 feet of sinks. Connection points that are not readily accessible or those for appliances occupying dedicated space, which are cord-and-plug connected in accordance with NEC Section 400-7A(6-8), are exempted.

The laboratory should be fitted with an adequate number of electrical outlets, which can accommodate electrical current requirements with an additional 20-40 % capacity.

Circuit breakers should be located outside the laboratory, but not in rated corridors

3. Laboratory ventilation and Fume hoods.

The primary functions of ventilation systems are to provide a comfortable, safe, breathable environment for all employees and public and to minimize the exposures to hazardous air contaminants. Careful planning, designing and maintaining air supply and exhaust vents and equipment and accomplish these goals.

3.1 General Requirements

All laboratory spaces shall have 100% outside air and exhaust to the outside.

There shall be no returns of fume hood and exhaust back into the building, of the order of 5-10 air changes per hour of ventilation for laboratory is desired.

Fume hoods shall not be the sole means of room air exhaust.

General room exhaust outlets shall be provided wherever necessary.

Adequate numbers and types of fume hoods shall be installed.

The system shall have at least 25% excess capacity for future expansion.

The noise generated by the mechanical devices shall be kept low

The laboratory windows, if they are to be opened, must be fitted with insect screens.

4. Emergency eye wash and Safety shower.

Eyewash and shower equipment for emergency treatment of the eyes or body of a person who has been exposed to chemicals, must be incorporated into the laboratory design

A plumbed eyewash and safety shower meeting the specifications of ANSI, z358.1, shall be provided.

An emergency shower combined with eyewash shall be provided at all work areas where, materials which are corrosive or otherwise hazardous on bodily contact are used.

A combination unit shall be installed with in all acid washing work areas.

An emergency eyewash or shower station shall be located near to the hazards area and there shall be a free path to reach the unit.

The water from the eyewash or shower shall be delivered at a tepid temperature and the pipeline shall be controlled by a ball-type shutoff valve which is visible, well mark and freely accessible.

The area of the shower shall be painted with a bright colour and shall be well lit.

5. Pressure vessel components and compressed gas cylinders.

Cylinders of compressed gases shall be stored in areas where they are protected from external heat sources. They must always be secured to prevent being knocked over.

Cylinders shall not be kept in unventilated enclosures such as lockers and cupboards.

Wherever practicable cylinders of compressed gas shall not be stored in laboratory areas. They should be located outside the building and supplies piped in. Flammable gases should have flame arrestors fitted to the cylinder

Adequate space shall be made available for the segregation of gases by hazard class.

Liquefied fuel-gas cylinders shall be stored in an upright position.

Storage area shall be secured against unauthorized entry.

Emergency power shall be provided for exhaust ventilation, gas leakage detection system and emergency alarm system.

6. Hazardous material storage cabinets.

Laboratories that store, use, or handle more than 10 gallons of flammable or combustible liquids shall have one or more flammable liquid storage cabinets.

Proper ventilation shall be provided for the storage cabinets and toxic materials shall be properly anchored to prevent spillage.

Secure, managed and monitored storage must be provided for highly toxic materials, e.g. cyanides, micotoxins, barium salts.

7. Model Floor Plans.

Model Floor Plan for Drug Lab :

i. For 4 Persons

ii For 6 Persons

iii Instrumentation Room

iv Safety Hoods Room

8. Relevant Links.

Links to laboratory Design.

<http://www.wbdg.org/>

<http://www.laboratorydesign.com/>

3. Equipment and Consumables

1. MANAGEMENT OF EQUIPMENT

Equipment management is a key issue for all laboratories. It covers purchasing, installation, documentation, availability, calibration, maintenance and servicing.

All laboratories should have reference standards of measurement appropriate to their activities. These may be as basic as a calibrated thermometer and a set of reference masses or they may extend across many physical quantities.

Traceability of calibration of equipment is a somewhat misunderstood subject but, for ISO 17025 accreditation purposes, all laboratories must be able to demonstrate that the measurements they make are made with instruments whose calibration can be traced back to the fundamental SI unit of measurement. This traceability chain may be through a number of steps each of which increases the uncertainty of measurement but, provided the measurement at the end of the chain is fit for its purpose, the measurement is regarded as traceable. Each step in the calibration chain must be documented and technically credible. Typically the laboratory will enter the traceability chain by having its equipment or references calibrated against national standards.

In addition to standards of measurement, laboratories will have some general equipment, common to most laboratories working in their field and also have equipment which is unique to the tests they perform and which is normally specified in the test methods. In all cases, a laboratory must provide an environment that ensures the equipment is protected from corrosion, excessive dust, vibration and other factors which could lead to its failure, loss of calibration or deterioration.

Equipment must be maintained at a satisfactory level of performance. For large items this may mean regular servicing by competent service personnel and regular calibration against higher level standards either by laboratory staff or by external specialists. The requirement is that whoever performs this calibration work must be demonstrably competent and must use properly calibrated standards to ensure continued measurement traceability. This can be achieved by in-house training of staff or by the use of an external, accredited, calibration service.

Equipment that has been damaged or is otherwise becomes unfit for service, e.g. out of calibration, must be clearly identified as such. Laboratories are encouraged to adopt sound preventive maintenance practices and, where possible to carry out regular checks on equipment so as to minimize the possibilities for use of unserviceable or out of calibration equipment.

Instruction manuals should be maintained and readily available to all operational staff. Laboratory reference materials and other reference standards of measurement, must be treated with care. They must be stored under conditions that will ensure their stability and the maintenance of their specified characteristics for their expected life. They must be

clearly identified with proper records of their history and performance. When appropriate, they should be re-verified, downgraded or discarded at the end of their certified life.

Reference materials for use in chemical measurements consist of substances of certified purity, often supplied as solutions of certified concentration. Additionally there are 'matrix reference materials' which are samples similar to those typically tested in the laboratory but with certified values of one or more test parameters. These materials enable the laboratory to check the accuracy of its measurements.

Policy for dealing with reference materials is a difficult area for accreditation bodies as there is, as yet, no international system for their verification nor is there a transparent mechanism to provide users with objective evidence that particular materials are what they purport to be. At the moment there are a number of reference material producers and suppliers that have earned a reputation for providing well-characterized materials with reliable documentation but acceptance or rejection based on reputation alone is a dangerous practice.

ILAC and its members are currently examining possibilities for accreditation of reference material providers to give some objective evidence that these materials are of a satisfactory standard and to enhance the transparency of the process. ILAC Guideline document G12 expresses its current thinking on the subject.

Laboratory equipment refers to the various tools and instruments used by scientists working in a laboratory. These include items such as balances, microscopes and specialty equipment such as spectrophotometers, calorimeters, chromatographs etc. Laboratory equipment is generally used to either perform an experiment or to take measurements and gather data. Some of the factors to be considered in choosing equipment are discussed in detail below.

1. Criteria for equipment selection
2. Technology and Laboratories
3. Equipment Qualification
4. Major Equipment.
5. Support Equipment.

1.Criteria for equipment selection

The criteria of selection of equipment should include suitability for the application, cost per test, the effect on personnel, the effect on provisions and facilities, the effect on environment, and the effect of possible outsourcing to a referral laboratory. There should be a system for evaluation of capital expenditure including direct and indirect (staffing) costs. The appropriate laboratory specialist from an accredited laboratory should be responsible for the selection criteria for the procurement of equipment, materials and reagents

The laboratory should be furnished with all items of equipment required for the provision of services (including primary sample collection, sample preparation and processing,

examination and storage). In those cases where the laboratory needs to use equipment outside its permanent control, laboratory management should ensure that the same requirements are met.

2. Technology and Laboratories

Production of laboratory equipment is generally a commercial activity carried out by a relatively small number of large instrument manufacturers in most cases. In some areas where specialist equipment is required, e.g. textile testing, petroleum products, building materials, there may be only two or three possible suppliers.

Innovations generally arise from research and development either within the equipment manufacturing sector or derived from academic research. Often there is a cooperative effort with academic institutions being sponsored by the industry.

The analytical laboratory needs to remain abreast of developments relevant to its area of operations as innovations may not only enable it to improve its performance but may also provide opportunities for more cost effective approaches.

3. Equipment Qualification

It is a basic requirement of a good analytical laboratory that all its analytical instruments must be suitable for the purpose to which they are applied and appropriately calibrated. As a consequence, Equipment Qualification gains more and more importance in ensuring the validity of results. Regulatory bodies also seem to be increasingly turning their attention to this area and manufacturers of analytical equipment are forced to take a significant part in the different steps of equipment qualification. There are four main steps in equipment qualification

- A. Design Qualification (DQ)
- B. Installation Qualification (IQ)
- C. Operational Qualification (OQ)
- D. Performance Qualification (PQ)
- E. Maintenance Qualification (MQ)
- F. Equipment Qualification process and user responsibility matrix
- G. Relevant links

A. Design Qualification (DQ) - defines the functional and operational specifications of equipment. As part the design qualification, the vendor should be qualified. Vendor Qualification may include good reputation, own experience, good references from other users, certification of the vendor such as ISO-9001, after sales service and availability of spares. A vendor qualification check list with the following points may be prepared.

- i. Does the vendor have a documented Quality System ?
- ii. Is the equipment hardware and computer software developed and validated according to a documented procedure?

iii. Is the vendor prepared to make product development and validation records accessible to regulatory agencies ?

iv. Does the vendor provide assistance in DQ,IQ,OQ maintenance and timely repair through qualified employees. Send the checklist to the supplier for his answers. If the supplier does not provide satisfactory answers, look for another supplier.

B. Installation Qualification (IQ) - ensures that equipment is received and installed as identified in the DQ and as specified in the order placed. IQ documents the installation in the selected user environment. Ensure the following is checked and recorded before and during installation.

i. Before Installation : - 1. Obtain manufacture's recommendation for installation site requirements.

2. Check the site for compatibility with the manufacturer's requirements. Consider especially electricity, water, gas, humidity, temperature etc.

3. Allow sufficient space for the equipment itself, spares storage and documentation such as related SOPS, operating manuals, log books and software.

ii. During Installation :

1. Compare equipment, as received with purchase order (including software ,spares, accessories etc.)

2. Check documentation for completeness (operating manuals, maintenance instruments etc.)

3. Install hardware

4. Switch on the instruments and ensure equipment powers- up.

5. Install software and computer following manufacture's recommendation.

6. Make back-up copy of software

7. Configure peripherals

8. Identify hardware and list in log book

9. List equipment manuals and SOPs required

10. Prepare installation report .

The following information, as a minimum, should be recorded for each piece of equipment. This can be kept as hard copy or on computer.

a. In-house Id number

b. Name of Item or equipment.

c. Manufacturer's name, address, phone number, service contract reference

d. Serial member of equipment.

e. Computer hardware with information on processor

f. Installed software and its revision member

g. Date received

h. Date placed in service .

i. Correct location

j. Conditions when received.

k. List of authorized users and responsible person.

C. Operational Qualification (OQ) - demonstrates that the equipment will function according to its operational specification in the selected environment . Before Operational Qualification is being performed , it is necessary to have answers for the following:

- i. Procedure and list standards to be used to verify performance. These may be specific to a particular intended use of the equipment or may reflect general performance for equipment with broad application. For example a balance will be generally checked with reference weights whereas a chromatograph purchased for a particular determination will be checked against the requirements of that analysis.
- ii. Acceptance criteria - should require performance as specified by manufacturer as a minimum plus any other criteria specific to the intended use.
- iii. OQ plan including frequency of OQ.
- iv. Who will perform OQ - vendor or user.
- v. Minimum re-qualification requirements if equipment is moved or after any repairs.

D. Performance Qualification (PQ) - demonstrates that the equipment consistently performs according to a specification appropriate for its routine use. PQ shall always be performed under conditions that are similar to routine sample analysis. The equipment shall perform consistently . The test frequency not only depends on the stability of the equipment but on every thing in this system that may contribute to the analysis. In other words PQ can be treated as system suitability test .

- i. Define the performance criteria and test performance.
- ii. Select critical parameters.
- iii. Define test intervals.
- iv. Define corrective actions.

E . Maintenance Qualification (MQ) The MQ describes and documents any maintenance required on the equipment. This includes routine servicing and any repairs necessary. Details of any maintenance contracts are also documented in this section, together with a list of authorized service engineers. In addition, the MQ includes the routine cleaning of the equipment and also its ultimate disposal

F. Equipment Qualification process and user responsibility matrix :

Design Qualification Installation Qualification Operational Qualification Performance Qualification

Describe intended use of equipment Compare equipment with PO Develop SOP for OQ
Select service provider
Select equipment Check for damage Set time intervals and acceptance criteria Define how to detect, record and handle errors
Select supplier Completeness of documentation Completeness of documentation
Maintain log book

G. Links for further information. <http://www.labcompliance.com>
http://www.pharmainfo.net/pharmaceutical_company/equipment_validation/laboratory_equipment_Qualification
<http://www.chemistry.org/>
<http://www.iir-events.com/>

<http://www.management-forum.co.uk/>
<http://www.journals.cambridge.org/>

4. Major Equipment

- A. Balance
- B. Spectrophotometry
- C. Gas-liquid chromatography
- D. High performance liquid chromatography
- E. Spectroscopy

A. Balance

Laboratories will have several balances and these are basic to most tests so their proper use, maintenance and verification is vital.

Using an Analytical Balance

Don't lean on the bench while weighing

Check the level indicator bubble on the balance before weighing and level the balance if necessary.

Remove any residues or dust on the balance pan or in the case.

Turn on the balance. The display lights up for several seconds, then resets to 0.0000.

Many balances will also go through an internal check and calibration procedure.

Never weigh directly on the balance pan. Place a suitable receptacle on the pan such as a weighing boat or at least a piece of paper.

Re-zero the balance with the tare control. The display should return to zero. If the balance is enclosed close the doors before taring.

Carefully add the substance to be weighed up to the desired mass. Do not attempt to reach a particular mass exactly.

Before recording the mass close any doors on the balance and wait until the reading is stable. Many balances will either beep or show a stability indicator.

If necessary record the weight on the sample container but also record the weight in your workbook or work sheet. The container will be discarded eventually !

Clean-up

Clean up any spillages on the balance immediately.

B. Spectrophotometry

A Spectrophotometer is a device that can measure intensity of light as a function of the colour, or more specifically, the wavelength of light. There are many kinds of Spectrophotometers. Among the most important distinctions used to classify them are the wavelength ranges over which they operate. In routine chemical analysis the ultra-violet/visible spectrometer is one of the commonest type of instrument and measures absorbance over wavelengths from 200nm to 750 nm typically. Infra-red spectrometers

which operate from around 800 to 4000 cm^{-1} are also applied in some areas notably pharmaceuticals.

Design There are two major classes of spectrophotometers; single beam and double beam. A double beam spectrophotometer measures the ratio of the light intensity on two different light paths only one of which passes through the sample. The other path passes through a reference such as a blank a single beam spectrophotometer measures the absolute light intensity of a single beam passing through the sample. Ratio (double beam) measurements are highly stable and free of background but require more expensive equipment. For most applications the simpler, cheaper and more compact single beam instruments are perfectly adequate.

Historically spectrometers used a grating or prism to separate wavelengths and then rotated it to bring each wavelength in turn to the detector. Modern instruments, however, generally have a fixed grating and measure all wavelengths at once using an array of semiconductor detectors. Infra-red spectrometers in particular adopt a completely different technique where the spectrum is analysed as a Fourier transform.

Irrespective of the details of the technique an absorption spectrophotometer measures quantitatively the fraction of light that passes through a given solution at one or more wavelengths. If any substance is present which absorbs at a particular wavelength then the light transmitted at that wavelength will be reduced and this reduction can be used to measure the amount of substance present by comparison with a series of standards. For this wavelength is then calculated.

In short, the sequence of events in a spectrophotometer is as follows:

1. The light source shines through the sample.
2. The sample absorbs light.
3. The detector detects how much light the sample has absorbed.
4. The detector then converts how much light the sample absorbed into a concentration based on data derived from running solutions of known concentration.

The numbers are either plotted straight away, or are transmitted to a computer to be further manipulated (e.g. curve smoothing, baseline correction).

Absorption spectrometry (often called colorimetry when use in the visible region) has a wide range of application. Any substance with a characteristic absorption wavelength can be measured directly but, in addition, any substance which can be subjected to a chemical reaction which generates a product which has a characteristic absorbing wavelength can also be measured indirectly. For example many metals form coloured complexes with organic reagents.

C. Gas-liquid chromatography

Gas-liquid chromatography (GLC), or simply gas chromatography (GC), is a type of chromatography, normally applied to organic compounds, in which the mobile phase is a carrier gas such as helium or nitrogen, and the stationary phase is a microscopic layer of liquid on an inert solid support, inside glass or metal tubing, called a column. Volatile materials are separated on the basis of the time they take to pass through the column. Broadly speaking this is determined by volatility so the lower boiling compounds emerge first. The eluted compounds are passed to a detector which records the area of the peak generated. By running solutions of known concentration a calibration curve can be constructed to enable the peak areas to be converted into concentrations.

Various types of detectors enable selective identification of the compounds separated. The most comprehensive such detector is the mass spectrometer which enables concentrations to be measured, allows the confirmation of identity of the materials and even the identification of unknowns.

D. High performance liquid chromatography

High performance liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry. The mobile phase is a liquid which may be anything from an organic solvent to an aqueous buffer. The stationary phase consists of a bed of fine particles usually based on silica but often highly modified by attaching functional groups. HPLC is used to separate components of a mixture by using a variety of chemical interactions between the substance being analyzed (analyte) and the functional groups on the chromatography column.

Detectors used in liquid chromatography include ultra-violet absorption, electrochemical, fluorescence spectrometers and refractive index. Liquid chromatographs can also be interfaced with mass spectrometers to provide selective detection and identification of unknowns.

E. Spectroscopy

Spectroscopy is the study of matter by investigating light, sound, or particles that are emitted, absorbed or scattered by the matter under investigation.

Spectroscopy may also be defined as the study of the interaction between light and matter. Historically, spectroscopy referred to a branch of science in which visible light was used for theoretical studies on the structure of matter and for qualitative and quantitative analyses. Recently, however, the definition has broadened as new techniques have been developed that utilize not only visible light, but many other forms of electromagnetic and non-electromagnetic radiation: microwaves, radio waves, x-rays, electrons, phonons (sound waves) and others.

Fluorescence spectroscopy: Fluorescence spectroscopy uses higher energy photons to excite a sample, which will then emit lower energy photons. This technique has become popular for its biochemical and medical applications, and can be used for confocal microscopy, fluorescence resonance energy transfer, and fluorescence lifetime imaging

X-ray spectroscopy and X-ray crystallography

When X-rays of sufficient frequency (energy) interact with a substance, inner shell electrons in the atom are excited to outer empty orbital's, or they may be removed completely, ionizing the atom. The inner shell "hole" will then be filled by electrons from outer orbital's. The energy available in this de-excitation process is emitted as radiation (fluorescence) or will remove other less-bound electrons from the atom (Auger effect). The absorption or emission frequencies (energies) are characteristic of the specific atom. In addition, for a specific atom small frequency (energy) variations occur which are characteristic of the chemical bonding. With a suitable apparatus, these characteristic X-ray frequencies or Auger electron energies can be measured. X-ray absorption and emission spectroscopy is used in chemistry and material sciences to determine elemental composition and chemical bonding.

X-ray crystallography is a scattering process; crystalline materials scatter X-rays at well-defined angles. If the wavelength of the incident X-rays is known, this allows calculation of the distances between planes of atoms within the crystal. The intensities of the scattered X-rays give information about the atomic positions and allow the arrangement of the atoms within the crystal structure to be calculated.

Atomic Emission Spectroscopy

Atomic emission spectroscopy uses a high temperature flame (e.g. air-acetylene), an electric arc or an argon plasma to excite atoms to higher electronic states. When the atoms relax to the ground state they emit light of a wavelength characteristic of the atom. These wavelengths can be separated by a prism or diffraction grating and their intensity measured.

By running solutions of known concentration the intensities can be converted into concentrations. Examples of this technique include inductively coupled plasma emission spectrometry which is discussed below.

Atomic absorption spectroscopy

In this technique for metals analysis samples, usually aqueous solutions, are passed either into a flame or a graphite furnace systems. This desolvates the sample and decompose any compounds. The result is free atoms of elements derived from the sample suspended in a cloud in the furnace or mixed in the flame. The temperatures used are such that the atoms are not excited as in emission spectroscopy, but they remain in the ground state. The concentration of the atoms is then measured by absorption spectroscopy at the characteristic wavelength for the atom. The light used is derived from lamps designed to emit the relevant atomic lines.

By calibrating with solutions of metals of known concentration the intensity of the atomic absorption can be converted into concentration units.

Atomic Fluorescence Spectroscopy

This method commonly uses a burner with a round burning outlet. The flame is used to solvate and atomize the sample, but a lamp shines light at a specific wavelength into the flame to excite the analyte atoms in the flame. The atoms of certain elements can then fluoresce emitting light in a different direction. The intensity of this fluorescing light is used for quantifying the amount of analyte element in the sample. A graphite furnace can also be used for atomic fluorescence spectroscopy. This method is not as commonly used as atomic absorption or plasma emission spectroscopy

ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectrometer)

This is the main type of atomic emission spectrometry in use in modern laboratories. As discussed above. It uses a radiofrequency powered argon plasma to produce a high temperature. A solution introduced into the plasma leads to excited atoms that emit electromagnetic radiation at a wavelength characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

In an increasingly applied variant the ions of the excited atoms are passed to a mass spectrometer (ICP-MS). This provides much lower detection limits than optical emission ICP.

Spark or arc (emission) spectroscopy - is used for the analysis of metallic elements in solid samples. For non-conductive materials, a sample is ground with graphite powder to make it conductive. In traditional arc spectroscopy methods, a sample of the solid was commonly ground up and destroyed during analysis. An electric arc or spark is passed through the sample, heating the sample to a high temperature to excite the atoms in it. The excited analyte atoms glow emitting light at various wavelengths which could be detected by common spectroscopic methods. Since the conditions producing the arc emission typically are not controlled quantitatively, the analysis for the elements is qualitative. Nowadays, the spark sources with controlled discharges under an argon atmosphere allow that this method can be considered eminently quantitative, and its use is widely expanded worldwide through production control laboratories of foundries and steel mills.

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy analyzes certain atomic nuclei to determine different local environments of hydrogen, carbon, or other atoms in the molecule of an organic compound or other compound. This is used to help determine the structure of the compound.

Photoemission spectroscopy

Photoemission Spectroscopy refers to two separate techniques

- a) X-Ray Photoemission Spectroscopy (XPS, formerly known as ESCA - Electron Spectroscopy for Chemical Analysis)
- b) Photoemission Spectroscopy (PES) may also refer to a technique devised by Richard Smalley . With Smalley's technique, X-rays are not used, but instead an UV laser is used to excite the sample. This method is designed primarily to find the binding energy of electrons in molecular clusters.

EQUIPMENT BASED ON SPECTROSCOPY PRINCIPLES:

FTIR, AAS, Fluorescence, OES/ICP, XRF, NMR etc.

Spares and Consumables : Ultra pure gases, Calibration standards/ CRMs, different wave-length specific lamps for AAS, ultra pure acids, sample digesters, Teflon tubing's as per equipment specification.

5. Support Equipment

- i. Burette
- ii. Pipette
- iii. Beakers
- iv. Volumetric Flasks
- v. Quantitative Transfer
- vi. Titration
- vii. Vacuum Filtration
- viii. pH Meter
- ix. Calorimeter
- x. Thermometer
- xi. Fume hood
- xii. Oven
- xiii. Furnace

- xiv. Magnetic stirrer / Mixer
- xv. Centrifuge
- xvi. Temperature / Humidity Cabinets (Environmental chambers)
- xvii. Micropipettete
- xviii. Refractometer
- xix. Tintometer

i. Burettete

A burettete (also burette) is a vertical cylindrical piece of laboratory glassware with a volumetric graduation on its full length and a precision tap, or stopcock, on the bottom. It is used to dispense known amounts of a liquid reagent in experiments for which such precision is necessary, such as a titration experiment. Burettetes are extremely precise: class A burettetes are accurate to $\pm 0.05\text{mL}$.

Using a burettete

Burettetes require careful use to avoid systematic errors. When reading a burettete, the viewer's eyes must be at the level of the graduation to avoid parallax error. Even the thickness of the lines printed on the burettete matters; the bottom of the meniscus of the liquid should be touching the top of the line you wish to measure from. A common rule of thumb is to add 0.02mL if the bottom of the meniscus is touching the bottom of the line. Due to the precision of the burettete, even a single drop of liquid hanging from the bottom of a burettete should be transferred to the receiving flask, usually by touching the drop to the side of the receiving flask and washing into the solution with the experimental solvent (usually water). Through careful control of the stopcock and rinsing, even partial drops of liquid can be added to the receiving flask.

Tips to fill and use

To fill a burettete, close the stopcock at the bottom and use a funnel. You may need to lift up on the funnel slightly, to allow the solution to flow in freely.

You can also fill a burettete using a disposable transfer pipettete. This works better than a funnel for the small, 10mL bursts. Be sure the transfer pipettete is dry or conditioned with the titrant, so the concentration of solution will not be changed.

Before titrating, condition the burettete with titrant solution and check that the burettete is flowing freely. To condition a piece of glassware, rinse it so that all surfaces are coated with solution, then drain. Conditioning two or three times will insure that the concentration of titrant is not changed by a stray drop of water.

Check the tip of the burettete for an air bubble. To remove an air bubble, whack the side of the burettete tip while solution is flowing. If an air bubble is present during a titration, volume readings may be in error.

Rinse the tip of the burette with water from a wash bottle and dry it carefully. After a minute, check for solution on the tip to see if your burette is leaking. The tip should be clean and dry before you take an initial volume reading.

When your burette is conditioned and filled, with no air bubbles or leaks, take an initial volume reading. A burette reading card with a black rectangle can help you to take a more accurate reading. Read the bottom of the meniscus. Be sure your eye is at the level of meniscus, not above or below. Reading from an angle, rather than straight on, results in a parallax error.

Deliver solution to the titration flask by turning the stopcock. The solution should be delivered quickly until a couple of mL from the endpoint.

The endpoint should be approached slowly, a drop at a time. Use a wash bottle to rinse the tip of the burette and the sides of the flask. Your TA can show you how to deliver a partial drop of solution, when near the endpoint

ii. Pipette

A pipette is used to measure small amounts of solution very accurately. A pipette bulb is used to draw solution into the pipette.

Using a Pipette

Start by squeezing the bulb in your preferred hand. Then place the bulb on the flat end of the Pipette.

Place the tip of the Pipette in the solution and release your grip on the bulb to pull solution into the Pipette. Draw solution in above the mark on the neck of the pipette. If the volume of the pipette is larger than the volume of the pipette bulb, you may need to remove the bulb from the pipette and squeeze it and replace it on the pipette a second time, to fill the pipette volume completely

Quickly, remove the pipette bulb and put your index finger on the end of the pipette. Gently release the seal made by your finger until the level of the solution meniscus exactly lines up with the mark on the pipette. Practice this with water until you are able to use the pipette and bulb consistently and accurately.

Repipette

A repipette is a hand operated pump that dispenses solution. Its volumes are accurate to within about 2%. If you need a more precise volume measurement, remeasure at your bench using a more precise piece of glassware, like a graduated cylinder, pipette, or burette. Place a clean, empty container at the outlet. Pull up the top of the repipette as far as it will go Push down gently and completely to deliver the full amount of solution

iii. Beakers and Erlenmeyer Flasks

Erlenmeyer flasks and beakers are used for mixing, transporting, and reacting, but not for accurate measurements. The volumes stamped on the sides are for guidance only and must never be used for measurement.

Graduated cylinders are useful for measuring liquid volumes to within about 1%. They are for general purpose use, but not for quantitative analysis. If greater accuracy is needed, use a pipette or volumetric flask

iv. Volumetric Flasks

A volumetric flask is used to make up a solution of fixed volume very accurately. Volumetric flasks are supplied in specified tolerances.

To make up a solution, first dissolve the solid material completely, in less water than required to fill the flask to the mark. After the solid is completely dissolved, very carefully fill the flask to the 500mL mark. Move your eye to the level of the mark on the neck of the flask and line it up so that the circle around the neck looks like a line, not an ellipse. Then add distilled water a drop at a time until the bottom of the meniscus lines up exactly with the mark on the neck of the flask. Take care that no drops of liquid are in the neck of the flask above the mark. After the final dilution, remember to mix your solution thoroughly, by inverting the flask and shaking.

v. Quantitative Transfer

Quantitative Transfer simply means that all the material to be transferred from one place to another must make the trip. For example, every particle of solid must be transferred from the weighing paper to the (clean) beaker.

This can be done by carefully tipping the creased weighing paper to pour the solid into the beaker. Tapping the paper with a spatula will knock particles into the beaker.

Finally, the paper should be rinsed into the beaker, to remove all traces of the solid.

Transferring a Solution or Mixture

If you are transferring a solution or heterogeneous mixture to another vessel, rinse the container with solvent to be sure the transfer is quantitative. The rinsing should be transferred to the second vessel along with the rest of the mixture or solution.

vi. Titration

A titration is a method of analysis that will allow you to determine the precise endpoint of a reaction and therefore the precise quantity of reactant in the titration flask. A burette is used to deliver the second reactant to the flask and an indicator or pH Meter is used to detect the endpoint of the reaction.

Doing a Titration

Begin by preparing your burette, as described on the burette page. Your burette should be conditioned and filled with titrant solution. You should check for air bubbles and leaks, before proceeding with the titration.

Take an initial volume reading and record it in your notebook. Before beginning a titration, you should always calculate the expected endpoint volume.

Prepare the solution to be analyzed by placing it in a clean Erlenmeyer flask or beaker. If your sample is a solid, make sure it is completely dissolved. Put a magnetic stirrer in the flask and add indicator.

Use the burette to deliver a stream of titrant to within a couple of mL of your expected endpoint. You will see the indicator change colour when the titrant hits the solution in the flask, but the colour change disappears upon stirring.

Approach the endpoint more slowly and watch the color of your flask carefully. Use a wash bottle to rinse the sides of the flask and the tip of the burette, to be sure all titrant is mixed in the flask. As you approach the endpoint, you may need to add a partial drop of titrant. You can do this with a rapid spin of a Teflon stopcock or by partially opening the

stopcock and rinsing the partial drop into the flask with a wash bottle. Ask your TA to demonstrate these techniques for you, in the lab.

Make sure you know what the endpoint should look like. For phenolphthalein, the endpoint is the first permanent pale pink. The pale pink fades in 10 to 20 minutes.

If you think you might have reached the endpoint, you can record the volume reading and add another partial drop. Sometimes it is easier to tell when you have gone past the endpoint.

If the flask looks like this, you have gone too far!

When you have reached the endpoint, read the final volume in the burette and record it in your notebook.

Subtract the initial volume to determine the amount of titrant delivered. Use this, the concentration of the titrant, and the stoichiometry of the titration reaction to calculate the number of moles of reactant in your analyte solution.

Titration with a pH meter follows the same procedure as a titration with an indicator, except that the endpoint is detected by a rapid change in pH, rather than the colour change of an indicator. Arrange the sample, stirrer, burette, and pH meter electrode so that you can read the pH and operate the burette with ease.

To detect the endpoint accurately, record pH vs. volume of titrant added and plot the titration curve as you titrate.

vii. Vacuum Filtration

Vacuum filtration is a technique for separating a solid product from a solvent or liquid reaction mixture. The mixture of solid and liquid is poured through a filter paper in a Buchner funnel. The solid is trapped by the filter and the liquid is drawn through the funnel into the flask below, by a vacuum.

Setting up and performing a vacuum filtration

To prepare for a vacuum filtration, gather together a filter flask, Buchner funnel, tubing, filter paper, clean solvent, disposable dropper, and your sample. A plastic or glass container should be interspersed between the filter flask and the vacuum system. This container prevents solvent from being sucked into the central vacuum pump by mistake. The tubing should be connected from the vacuum nozzle to the trap and from the trap to the side arm of your filter flask. Turn on the vacuum. Check the vacuum by feeling for suction at the end of your tubing. The vacuum should be strong enough to hold the tubing to your finger without falling off. If you do not feel strong suction, there is a poor connection and a leak somewhere in your system.

viii. pH Meter

A pH meter uses an electrode to measure the pH of a solution.

Calibrating a pH Meter

Remove bottle with storage solution, rinse electrode, blot dry. Measure pH of 4 buffers, which is pink. Adjust meter to read 4 with Cal 1 knob on left. Remove pH 4 buffer, rinse electrode, blot dry. Measure pH of 10 buffers, which is blue. Adjust meter to read 10 with Cal 2 knob on right. Remove pH 10 buffer, rinse electrode, blot dry. Measure pH of 4 buffers again. pH should read 4. If not, readjust Cal 1 knob. Return to pH 10 buffer. pH should read 10. If not, readjust Cal 2 knob. Repeat the standardization using the Cal 1

knob with the pH 4 buffer and the Cal 2 knob with the pH 10 buffer until consistent readings are obtained.

ix. Calorimeter

Calorimetry is used to determine the heat released or absorbed in a chemical reaction. The calorimeters shown here can determine the heat of a solution reaction at constant (atmospheric) pressure. The calorimeter is a double Styrofoam cup fitted with a plastic top in which there is a hole for a thermometer. (It's crude, but very effective!) Key techniques for obtaining accurate results are starting with a dry calorimeter, measuring solution volumes precisely, and determining change in temperature accurately.

Using a Calorimeter

Solution volumes should be carefully measured with a graduated cylinder. Add solution completely, to a dry calorimeter. Don't forget to add the spin bar each time! Set up the calorimeter with the thermometer (0° to 50°C , graduated every 0.1°C) supported from a stand so that the bulb does not touch the bottom of the cup. Note that the thermometer used for calorimetry differs from the less accurate one in your glassware drawer. Clamp the calorimeter so that it rests on the stirrer. Be careful not to turn on the heat or you will melt the Styrofoam. The change in temperature is determined by measuring the initial temperature, T_1 , of the reactants, and the maximum temperature, T_2 , of the contents of the calorimeter during the exothermic reaction. Use a magnifying glass to measure temperature values precisely. Interpolate between the divisions of the thermometer and record temperatures to $\pm 0.01^{\circ}\text{C}$. See your lab manual for a discussion of how to determine accurately the change in temperature from your graph of temperature vs. time.

x. Thermometer

A thermometer is a device that measures temperature or temperature gradient, using a variety of different principles. The word thermometer is derived from two smaller word fragments: thermo from the Greek for heat and meter from Greek, meaning to measure. A thermometer has two important elements, the temperature sensor (e.g. the bulb on a mercury thermometer) in which some physical change occurs with temperature, plus some means of converting this physical change into a value (e.g. the scale on a mercury thermometer). Industrial thermometers commonly use electronic means to provide a digital display or input to a computer.

Types of thermometers

Thermometers have been built which utilize a range of physical effects to measure temperature. Most thermometers are originally calibrated to a constant-volume gas thermometer. Temperature sensors are used in a wide variety of scientific and engineering applications, especially measurement systems. Temperature systems are primarily either electrical or mechanical, occasionally inseparable from the system which they control (as in the case of a mercury thermometer).

- Alcohol thermometer
- Basal thermometer
- Mercury-in-glass thermometer
- Bi-metal mechanical thermometer
- Electrical resistance thermometer

- Galileo thermometer
- Infrared thermometer
- Liquid Crystal Thermometer
- Reversing thermometer
- Silicon bandgap temperature sensor
- Six's thermometer- also known as a Maximum minimum thermometer
- Thermistor
- Thermocouple
- Coulomb blockade thermometer
- Oral thermometer

Calibration:

Thermometers can be calibrated by placing them in different frigorific mixtures. A mixture of ice and pure water, for example, has an equilibrium temperature of 0 degrees Celsius. A mixture of ice, water and salt has an equilibrium temperature of -9 degrees Celsius (0 degrees Fahrenheit). Water that is boiling at sea level (a mixture of liquid water and water vapor) has an equilibrium temperature of 100 degrees Celsius. By placing a thermometer in each of these three mixtures, the thermometer can be calibrated. Calibration certificates issued by manufacturers often does not bear traceability and so are un-accepted by the assessors during audits. In such cases certificates from accredited calibration labs are to be procured well before audits

Appliance Thermometers:

Appliance thermometers are specially designed to measure the temperature of the air in either the refrigerator/freezer or the oven. Some refrigerator thermometers have long metal probes and are similar in appearance to food thermometers. Other refrigerator thermometers, and most oven thermometers, are designed to hang from a wire rack or sit on a shelf.

Whether they measure the temperature in the oven or refrigerator/freezer, most appliance thermometers are either liquid-filled or bimetallic-coil thermometers:

Liquid-filled Thermometers, also called "spirit-filled" or "liquid in glass" thermometers, are the oldest types of thermometers used in home kitchens. As the temperature increases, the colored liquid (usually an alcohol solution) inside the thermometer expands and rises to indicate the temperature on a scale.

Bimetallic-coil Thermometers contain a coil made of two different metals with different rates of expansion that are bonded together. The bimetal element is coiled, fixed at one end, and attached to a pointer stem at the other end. As the temperature increases, the pointer will be rotated by the coiled bimetal element to indicate the temperature.

Using Appliance Thermometers

Refrigerator/Freezer Thermometers

Refrigerator/freezer thermometers are specially designed to provide accuracy at cold temperatures. For safety, it is important to verify the temperature of refrigerators and freezers. Refrigerators should maintain a temperature no higher than 40°F. Frozen food will hold its top quality for the longest possible time when the freezer maintains 0°F. Most refrigerators and freezers can be easily adjusted to run colder or warmer. The temperature control is usually accessible in the refrigerator part of the appliance. Check

the owner's manual for specific details on adjusting the temperature. An adjustment period is often required when changing the temperature.

To measure the temperature in the refrigerator:

Put the thermometer in a glass of water and place in the middle of the refrigerator. Wait 5 to 8 hours. If the temperature is not 38 to 40°F, adjust the refrigerator temperature control. Check again after 5 to 8 hours.

To measure the temperature in the freezer:

Place the thermometer between frozen food packages. Wait 5 to 8 hours. If the temperature is not 0 to 2°F, adjust the freezer temperature control. Check again after 5 to 8 hours.

An appliance thermometer can be kept in the refrigerator and freezer to monitor the temperature at all times. This can be critical in the event of a power outage. When the power goes back on, if the refrigerator is still 40°F and the freezer is 0°F or below, the food is safe.

Oven Thermometers

An oven thermometer can be left in the oven to verify that the oven is heating to the desired temperatures. When cooking meat and poultry, it is important that the oven be set at 325°F or higher. These thermometers can measure temperatures from 100 to 600°F.

To check the accuracy of an oven, hang the oven thermometer from a rack in the center of the oven (you may have to adjust the oven racks). Set the oven for 325 to 350°F and allow it to preheat. Once the oven has reached the set temperature, open the oven door and read the thermometer. The oven maintains its temperature by cycling on and off, especially if the door has been opened. Check the temperature again after 5 minutes.

If the oven is not maintaining the set temperature, the oven thermostat will have to be adjusted by a service center representative authorized by the manufacturer. However, if, after testing the oven temperature at several settings (325, 350, 375, and 400°F), it is consistently high or low by the same amount (say, 25°F), this can be factored into the temperature setting. For example, if you know that your oven runs "hot" by 25°F and you need to bake something at 350°F, set the oven for 325°F. Always check the oven thermometer to verify the temperature.

Microwave Oven Probes

A microwave oven probe can be plugged into the microwave and inserted in the food being cooked. Some microwaves can be programmed to cook the food until a desired temperature is reached. Check the owner's manual for more information. Some thermometers are specially designed to be used in the microwave oven, but most food thermometers are not microwave-safe. Check the packaging instructions for more information.

xi. Fume hood

A fume hood or fume cupboard is a large piece of scientific equipment common to chemistry laboratories designed to limit a person's exposure to hazardous fumes. Fume hoods were originally manufactured from timber, but now epoxy coated mild steel is the main construction material. Two main types of unit exist, ducted and recirculating. With the ducted type, old asbestos vent pipe has been superseded on health grounds, typically with PVC or polypropylene. The principle is the same for all units; air is drawn in from

the front of the cabinet by a fan, and expelled either outside the building, or made safe through filtration and fed back into the room.

xii. Oven

An oven is an enclosed compartment for heating, baking or drying

xiii. Furnace

A furnace is a device used for heating.

The heat energy to fuel a furnace may be supplied directly by combustion of some fuel, or electric furnaces such as the electric arc furnace or induction furnace use remotely generated electric power

xiv. Magnetic stirrer / Mixer

A magnetic stirrer consists of a small bar magnet (or stir bar), which is normally coated in a plastic such as PTFE and a stand or plate containing a rotating magnet or stationary electromagnets creating a rotating magnetic field. Often, the plate can also be heated. During operation of a typical magnetic stirrer, the bar magnet (or flea) is placed in a vessel containing a liquid to be stirred. The vessel is set on top of the stand, where the rapidly rotating magnetic field causes the bar magnet to rotate.

xv. Centrifuge

A centrifuge is a piece of equipment, generally driven by a motor, that puts an object in rotation around a fixed axis, applying force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal force is used to separate lighter and heavier substances. There are many different kinds of centrifuges, including those for very specialized purposes.

Spares/ Consumables: Sample holders of different sizes, centrifuge tubes

xvi. Temperature / Humidity Cabinets (Environmental chambers)

Environmental test chambers are used to ensure the reliability of Drugs & Pharma. Products, industrial products, especially electronic items etc., through prolonged exposure to one or more environmental parameters. They provide heating and/or cooling and often include an analogue meter, digital readout, or video display with test results. Some environmental test chambers subject samples, components, or products to extreme temperatures, thermal cycling, or extreme humidity. Others monitor emissions from devices such as internal combustion engines under carefully controlled conditions. Chambers for the electro-dynamic and mechanical vibration testing of machinery are

available. Environmental test chambers that measure corrosion monitor the effects of variables such as heat, humidity, salt fog, salt spray, and rain spray.

Performance specifications for environmental test chambers include temperature range, temperature control accuracy, humidity range, humidity control accuracy, and chamber capacity. Temperature range and humidity range are full-scale values. Temperature range accuracy is measured in degrees or as a percentage of a full-scale output or reading. Humidity control accuracy is expressed as a percentage. Some environmental test chambers are designed to be used atop a desk, table, or work bench. Others are free standing and include hardware for floor-mounting. Reach-in chambers provide a window that users can open to handle test contents. Walk-in chambers include a full-size door. Environmental test room and laboratory-scale environmental test chambers are commonly available.

xvii. Micropipette

Micropipettes are micro tools constructed from glass tubings for microinjection and micromanipulation purposes. Many types and sizes of glass tubing are available, mainly in three different compositions: borosilicate, aluminosilicate and quartz. Each composition has its own unique properties and the right selection is determined by the application it is used for.

xviii. Refractometer

A refractometer is an optical instrument that is used to determine the refractive index of a substance. This often refers to some physical property of a substance that is directly related to its refractive index. Certain types of refractometers can be used for measuring gases, liquids such as oils or water-based, and even transparent or translucent solids such as gemstones.

A refractometer can be used to determine the identity of an unknown substance based on its refractive index, to assess the purity of a particular substance, or to determine the concentration of one substance dissolved in another. Most commonly, refractometers are used for measuring fluid concentrations such as the sugar content (Brix level, for example in carbonated beverages, fruits, juices, honey and or vegetables, etc), blood protein concentration, salinity and specific gravity of urine. Refractometers are also used for measuring fluid concentrations for commercial liquids such as antifreeze, cutting fluid, and industrial fluids.

Types of refractometers

There are four main types of refractometers: traditional handheld refractometers, digital handheld refractometers, laboratory or Abbe refractometers, and inline process refractometers.

There is also the Rayleigh Refractometer used (typically) for measuring the refractive indices of gases.

In veterinary medicine, a refractometer is used to measure the total plasma protein in a blood samples.

xix. Tintometer

It is a measuring instrument used in colorimetric analysis to determine the quantity of a substance from the color it yields with specific reagents

The Roth Amsted device is an obturator vane by which the observer, when matching a specimen by a combination of Lovibond glasses, can make the two surfaces he is viewing look equally bright. This device greatly reduces the number of combinations required for colour matching. Conversion graphs give the C.I.E. co-ordinates for these combinations in Standard Illuminant B. The C.I.E. co-ordinates of matched colors are thereby obtained.

4. Staff, skills and training

Staffing

It is necessary that all personnel recruited for the laboratory operations have basic qualifications, skills and necessary experience.

The management has to specify the minimum requirements and experience for each position, before recruitment of personnel.

An assessment needs to be done on the following areas to arrive at a conclusion regarding staff recruitment:

Scope of the laboratory activities

Magnitude / quantum of samples to be turned out in each section (present situation and future improvement)

Types of equipment and accessories to be handled.

National / International regulatory requirements

Skills

To illustrate the above, a model qualification and expertise requirements are given below:

DESIGNATION	TYPICAL QUALIFICATION & PROFESSIONAL CAPABILITY	SKILLS REQUIRED
Quality Manager / Quality Assurance Unit team member	Should be a Graduate in Science with approximately 2 years laboratory experience. - -Should have knowledge of relevant standards, quality audits, supervision, resolving customer complaints - -Knowledge of Procedures for recording, reporting, checking & interpretation of test results - -Familiarity with test & calibration procedures and awareness of limitations of these procedures	Managerial capabilities - Contract review - Equipment calibration requirements -Familiarity with Method validation, Proficiency testing and QC Checks, -Familiarity with Management system requirements -Conduct of audits in the lab -Awareness of the requirements and conditions for accreditation to ISO / IEC 17025 relating to test reports.
Head of Lab / Group Leader	-Should be a Post -Graduate in Science in relevant field with 5 year laboratory experience.	-Contract review - Equipment calibration requirements

	<ul style="list-style-type: none"> -Should have knowledge of relevant standards, protocol preparation, supervision, interpretation of test results and reporting -Knowledge of Procedures for recording, reporting and checking results -Familiarity with test and calibration procedures and awareness of limitations of these procedures 	<ul style="list-style-type: none"> -Design and validation of new methods, Measurement traceability & Uncertainty of measurement -Evaluation of training of personnel -Awareness of the requirements and conditions for accreditation to ISO/IEC 17025 relating to test reports
Scientist / Technical Assistant	<ul style="list-style-type: none"> -Should be a Post Graduate or Graduate in Science with laboratory or testing experience; Should have knowledge of relevant standards. -Familiarity with test procedures 	<ul style="list-style-type: none"> -Capability of performing the tests as per SOP / Specification -Capability of operating the instruments as per the requirement -Equipment calibration and maintenance requirements -Measurement traceability & Uncertainty of measurement -Use of standard methods & validation of new methods.

Training

Training is an essential part of the management system and a vital method for improvement:

This training procedure is used to ensure that training has taken place with each employee for procedures and methods that the employee performs. The procedure applies to on-the-job training, in-house training and new-hire training. The training is verified and documented. The training procedure is applicable to new employees, for the introduction of new procedures and methods, for retraining of employees, and for re-verification of employee performance.

Responsibilities of Staff at different levels
Second Level Manager

Ensures implementation of training procedure

Ensures resources are allocated for identified training within budgetary constraints

Responsible for the evaluation, training and growth of the technical- and quality related skills of employees by establishing training schedule and rotation for all new employees and by ensuring personnel receive training and demonstrate competence

Ensures training is accomplished

Submits documentation for completed training for entry into training database or Integrated Management of Personnel Administration through Computer Technology and

Identifies training needs and courses of personal under his supervision and make necessary schedule

First Level Manager

Implements training and maintains employee training files

Ensures proper supervision of trainees until training completed

Reviews training received and ensures training files are complete

submits documentation for training completed

Monitors employee performance to identify the need for retraining or additional continual education, and

Identifies training needs resulting from new or revised procedures and processes.

Quality Manager

Liaison with accreditation bodies.

Trains employees in quality control and quality assurance procedures.

In case of non -conforming tests, find out the root cause, identify critical areas. Implement corrective actions, identify preventive actions and implement verification of their implementation and effectiveness.

To convene Management Review committee meeting at scheduled time, record minutes and verify its implementation.

Identification of training needs of personnel and arrangement of conducting the training programs.

Planning and conducting internal audits and other quality assurance activity.

Approve, issue/ammendment and check the control of documents

Registration and redress of complaints.

Staff / trainees / junior scientists

Completes required training within specified timeframe;

Becomes Conversant and knowledgeable in procedures and methods performed. Employees are responsible for self-training, through reading current literature, technical papers, publishing technical papers;

Reports all training received and submits documentation for training received; and

Reads and complies with standards, regulations, policies, procedures,and work instructions.

Procedure

Before starting any work the employee should be familiar with all work related documents. These documents include procedures, work instructions, applicable manuals and regulations.

Training requirements are outlined and documented on the basis of the position,its duties and responsibilities.

The level of training is determined by the employee's educational qualifications, experience, complexity of the test method, and knowledge of the test method performed.

The employee will not perform any procedure, inspection, or method until all applicable training has been completed and competency demonstrated. Employees undergoing training are supervised until training is completed and competency demonstrated.

Employees may request training related to their job. Lab posts the training and development services for their staff on an annual basis. Included in these services are classroom courses, downlinks, lending library, certification and web-based courses. A model format for recording the above is given below.

S.No	Date	Location	Title of Training	Name of Trainee	Name of Trainer	Result of Assessment

Upon completion of training submit documentation such as sign-in sheets for entry into training database. Where evaluation is of capability to perform tests there must also be objective evidence of competence, e.g. results of satisfactory performance of test on reference materials.

Date	Details of Training Undergone	Signature of supervisor/Remarks

The effectiveness of training is evaluated by but not limited to reviews performed by management and performance evaluations.

TRAINING ASSESSMENT

Year: _____

Name of Employee: _____

Designation: _____

Sl.No.	Training program description	Trainer	Evaluation criteria * (Enclose details where applicable)	Assessment grade * * with date	Full signature of assessor / Head of Lab/ Supervisor

Note: Evaluation criteria shall be based on specific SOP/Standard requirements / Questionnaire. Provide proof of evaluation in terms of Knowledge gained (questionnaire & answers), Skills supervised, Test results, QC checks or competency in analyzing analyte(s) / testing as applicable.

Assessment grade:

A - Excellent (can extend training on the subject)

B - Satisfactory (able to implement at work the knowledge gained through training)

C - Unsatisfactory (requires retraining)

NA - If evaluation criteria and assessment are not applicable for certain training programmes

The training process for technical procedures such as laboratory analysis consists of the following steps:

1. Trainee reads the laboratory procedures, work instructions, or other applicable documents.

2. Trainee observes demonstration of the procedure by a trainer,
3. Trainee performs the procedure under observation by a trainer.
4. Trainee successfully completes the procedureM

Documentation of these tasks is submitted for filing in the employee's training file. The training process for non-technical procedures includes, but is not limited to:

1. Reading laboratory and other related National / International procedures.
2. Instructions,
3. Demonstrations,
4. Lectures and discussions,
5. Self study,
6. Computer-based training,
7. Viewing videotapes, and
8. Manufacturer's training or demonstration.

Demonstration of these tasks is submitted for filing in the employee's training file.

An employee's performance is verified by measurement against a defined performance standard. The measures used to verify an employee's performance are assessment tools.

Assessment tools include the following:

1. Administration of a Written Evaluation: Written evaluations can be used in areas where verification of a participant's knowledge is desired. Knowledge of theory of principles, problem-solving ability, logical sequence used and independent or group decision making may be ascertained.
2. Observation of procedure, process, or outcome: Observation by a trainer of an employee performing or demonstrating a procedure.
3. Verification of Response to Situational Problems or Calculations Related to the Procedure
4. Response to oral query related to a step or procedure: Answers provided by the employee to questions asked by trainer.
5. Testing blind QC samples: Employees are unaware when blind test samples are assigned. They appear identical to other samples, are in routinely used containers, and are from a similar source. The intent is to provide simulated samples to measure realistic analytic conditions. This tool assesses all phases of laboratory performance.
6. Testing of known samples : Participants know and often plan for known testing events, such as external proficiency surveys and commercially prepared quality control samples. Samples for quality assurance or quality control purposes are identified immediately upon receipt in the laboratory. It is considered a waste of time and resources to conduct

more careful handling and analysis on these samples or perform duplicate testing. This tool assesses the analytical phase only.

7. Testing previously analyzed samples: Duplicate or replicate testing provides accessible internal comparisons and contributes to the validation of the analytic phase. These sources may be previously tested samples, samples of known constituents, and already reported proficiency testing samples. This tool assesses the analytic phase only.

Documentation of Training Verification includes:

1. Completion of training checklists prepared internally for all procedures that an employee performs;
2. Completion of the procedure with submission of written evidence;
3. Completion of blind quality control (QC) samples, proficiency surveys, QC samples, and duplicate testing with submission of results that are within acceptable criteria limits;
4. Completion of written evaluations, signing acknowledgement of reading assigned work instructions;
5. Attendance sign-in sheets (See Attachment) on in-house training, certificates from manufacturer's training courses and computer classes, and committees served on;
6. Submission of technical papers and handouts of presentations given, college transcripts for courses taken, licenses and memberships held and special conferences attended
7. Completed paperwork on safety briefing, orientation modules, and in or out-processing steps for new hires or those leaving the organizational unit; and
8. Memorandums on additional appointments or duties.

Retraining

1. Employees will be re-trained whenever significant changes occur in policies, values, goals, procedures, processes, and methods or instruments.
2. Employees will be re-trained when the level of performance is unsatisfactory as shown by any of the assessment tools discussed above.

Re-verification

1. Re-verification occurs whenever employees attend required courses, continuing education, presentations, workshops, conferences and schedules training either in house or manufacturer's training.
2. Performance re-verification occurs whenever proficiency surveys, blind QC samples, or duplicate testing are submitted.

All analysts and laboratory staff members are to undergo training in a number of procedures, policies and policies upon entry of employment and during their career with the lab. What follows are types of required training:

1. Facility orientation includes:
 - i. New employees completing required administrative forms as part of initial processing; and
 - ii. Introduction to co-workers, personnel policies, working conditions, daily routine, issuance of manuals, quality assurance system and any miscellaneous matters.
2. New Training often includes:
 - i. Quality Manual and other policies.
 - ii. Management systems and ISO/IEC 17025 requirements.
 - iii. Quality Systems and Audit workshop,
 - iv. Investigative Interviewing course, and
 - v. Lab computer based training modules.
3. Safety Training may include the topics of :
 - i. Hazard communication standard (Right to Know)
 - ii. Universal precautions (e.g., hand washing and aids awareness),
 - iii. Medical surveillance program,
 - iv. Personal protective equipment,
 - v. Security briefing,
 - vi. Safety briefing,
 - vii. Fire extinguisher training,
 - viii. Emergency evacuation, and
 - ix. Safety practices in the laboratory
4. Chemical Hygiene
5. Hazardous Waste Management that includes annual training on handling, storage, and disposal of hazardous materials.

6. Quality Assurance including annual training on quality control (QC), quality assurance (QA), the Labs Quality Management System (QMS) and related procedures.
7. On-the-job training..
8. Training on policies, regulations, procedures, methods and instruments.

Often laboratory staff has an opportunity to attend auxiliary training when available and resources permitting. This type of training includes:

- i. Attendance at presentations, courses and conferences; and
- ii. Computer courses such as in-house training, instructional, and manufacturer's training on software in use such as Microsoft Word and Excel, Chem-Station, and Outlook.

5. Proficiency Testing

1. Introduction

One of the best ways for a laboratory is to monitor its performance it to participate in proficiency testing scheme. Proficiency testing is a type of inter-laboratory comparison exercise in which samples are circulated to the participating laboratories, results are then collated and evaluated centrally. Each laboratory is then informed of its performance relative to the other laboratories in the scheme and relative to either true or consensus result for the sample. Performance is usually quoted in terms of the number of standard deviations between the achieved value and the consensus or true value. In some schemes participants are given a pass /fail rating.

Because of the requirement of the laboratory accreditation bodies from all over the world more and more laboratories participate in proficiency testing schemes. Since in all fields of testing and calibration, the satisfactory performance by an applicant laboratory is the pre-requisite from the accreditation bodies, participation in PT is increasingly being used by laboratories. Moreover PT is being increasingly used by laboratories to demonstrate their competence and thus it is becoming more popular and acceptable to laboratories. Laboratories do face a problem as there are not adequate numbers of PT schemes to meet their need. While many laboratory accreditation bodies organize their own PT schemes many accreditation bodies do use PT schemes or other inter-laboratory comparison schemes being operated by others to evaluate the competence of their accredited laboratories.

To cope up with the increasing requirement for PT schemes or inter-laboratory comparison many commercial third party PT and inter-laboratory comparison providers are entering the market to fill the gap and thus meet the growing need. This has led to the need for maintaining a common standard for operating the inter-laboratory comparison programs. This need is met by operating the inter-laboratory comparison schemes as per the guidance given in ISO/IEC Guide 43 part I.

Some accreditation bodies have started giving accreditation to PT/ Inter-laboratory comparison program providers based on Guide 43.

2. Overall suitability of the design of specific proficiency testing program

4.1.1 ISO/IEC Guide 43 Part-1 and ISO/IEC Guide 43 Part-2, deal with all those issues which are technical & relating to the conduct of PT programs, such as "Development and operation of proficiency testing schemes" and "Selection and use of proficiency testing schemes by laboratory accreditation bodies" respectively.

4.1.2 APLAC MR 001 "Procedures for establishing and maintaining mutual recognition arrangements (MRA) amongst accreditation bodies" Includes the requirements which accreditation bodies are expected to apply when approving laboratory's arrangements for proficiency testing.

a. Proficiency testing activity (APLAC MR 001 Clause 3.3):

One PT activity prior to gaining accreditation

One PT activity relating to each other major sub-area of major disciplines of a laboratory's scope of accreditation at least every four years

ILAC-G13;2000 "Guidelines for the requirements for the competence of providers of PT schemes" This document spells its need for PT program.

4.1.3 ISO/IEC 17025 & PT(for laboratories) ISO/IEC 17025 has identified the following situations where application of PT or inter- laboratories comparisons are suggested.

- Test methods and methods validation (Clause 5.4 of ISO/IEC 17025) Validation of methods (clause 5.4.5.1 Note 2)

- Measurement traceability (Clause 5.6.2..2 of ISO/IEC 17025) Participation in a suitable program of inter-laboratory comparison, where required.

- Assuring the quality of test and calibration results, (Clause 5.9.1 b of ISO/IEC 17025))

Participation in inter-laboratory comparison or proficiency -testing programs

4.1.4 ISO/IEC 17011 on Proficiency testing(for accreditation bodies)

Clause 7.12.1 (ISO/IEC 17011) The accreditation body shall have and apply procedures to demonstrate the competence of its accredited laboratories by their satisfactory participation in proficiency testing activity, where such activities are available and appropriate. Where such activities are available and appropriate, the minimum amount of proficiency testing shall be specified. The amount of proficiency testing and frequency of participation have to be seen in relation to other surveillance activities.

Clause 7.12.2 (ISO/IEC 17011) The accreditation body may organize proficiency testing or other inter-laboratory comparisons itself or may involve another body, judged to be competent.

Clause 7.12.3 (ISO/IEC 17011) The accreditation body shall ensure that proficiency testing activities that its accredited or applicant laboratories have to undertake are

effective, linked to the assessment process and appropriate corrective action are carried out when necessary.

Clause 7.12.4 (ISO/IEC 17011) The accreditation body shall take into consideration, where relevant, the performance of laboratories in proficiency testing, when evaluating the competence of the staff and the validity of the results.

Clause 7.12.5 (ISO/IEC 17011) The accreditation body shall maintain a list of proficiency testing programs.

The agency which need to conduct PT programs is required to meet the obligation that may applicable to it. ISO/IEC Guide 43 I & II give the details for methodology to be followed in organizing a PT scheme.

3. Roles & uses of Proficiency Testing Programs

Determines the performance of the individual laboratory & monitors laboratory's performance

Identify problems in the laboratory and initiate remedial action

Establish effectiveness & comparability of non standard test and calibration methods, i.e. validation of a test method

Identify inter-laboratory differences

Assign values to the reference materials (RMs) and assess their suitability for use in specific test and measurement procedures

Establish traceability in measurements where calibrations are not possible or available and/or RMs/ CRMs are not available

4. Comparability of the test or measurement procedures used by each countries laboratories in a specific program

. Participants of a PT scheme normally use the method of test or calibration of their choice, which in consistence with the routine procedure being used by them. However in those cases where they have developed a test method or they intend to use a non-standard method they use a PT Program to validate.

In certain cases, a coordinator may ask the participants to use specified method. Such methods are usually internationally accepted standard methods.

Where a calibration procedure is used, the assigned value will often be a reference value obtained from a high -echelon calibration laboratory, National Standards Laboratory , and should use well defined accepted procedures.

Where participants are free to use a method of their choice, the coordinator may ask for details of method used to allow the use of participants results to compare and comment on the methods.

5. Types of PT scheme

Proficiency testing techniques vary depending on the nature of the test item, or material test method in use and the number of laboratories participating, or as per the requirement of client of lab and the regulatory authorities. The common feature in most of the PT programs is the comparison of test results obtained from participating laboratories using statistical methods. In some programs, one of the participating laboratories may be controlling and, coordinating. The most commonly used types of PT schemes are:

5.1 Measurement comparison schemes

Measurement comparison schemes involve circulation of the calibrated item/ artifact from one participating laboratory to the other, for the relevant measurement.

The reference value of the artefact is from a National Metrology Laboratory/Reference Laboratory, which is also in most of the cases is the coordinating laboratory and assigns values to the test item/artefact. This type of program may take a considerable time to complete the cycle of measurement. As the artefact is under circulation the coordinating laboratory ensures the integrity of the artefact.

Such an activity takes care of the range of measurement and also the uncertainty in measurements.

The individual measurement results are compared with the reference value established by the National Metrology Laboratory/Reference Laboratory. The coordinator is required to take into account the claimed measurement uncertainty of each participating laboratory. Items used (artefacts) in this type of proficiency testing are reference standards (resistors, gauges and instruments.). The path travelled by artefacts is depicted below:

Round robin type or circulation path

4.4.2 Inter-laboratory testing

Inter-laboratory testing schemes involve randomly selected sub-samples, demonstrated to be homogeneous, being distributed simultaneously to participating testing laboratories for concurrent testing. After completion of the testing, the results are returned to the coordinating body, and compared with the assigned value(s), to give an indication of the performance of the individual laboratories. The important features are:

Sub samples given to each laboratory are homogenous.

After completion of testing, results are returned to the coordinating laboratory and compare with an assigned values to give an indication of performance of the individual laboratory.

Items used in this type of PT are food, body fluids, water, soil and other environmental materials. Refer ISO/IEC Guide 43-1:1997.

Sub samples given to each laboratory are homogenous

6.Steps involved in organizing a PT program

Announcement of PT activity & inviting interest in PT participation from labs
Sending of samples to participating laboratory or ensuring for circulation of artefact amongst the participant labs
Receive the results from lab
Do the statistical analysis and send preliminary report to participating laboratories
Incorporation of feedback from laboratories
Sending final report to participating labs after receiving corrective action

7.Responsibilities & Authorities of the PT Program coordinator

PT Program coordinator shall have a PT committee with an expert on statistics and on the subject as its member besides other members with appropriate knowledge on the testing/calibration of the substance / item. In addition the body, shall:

Define responsibilities and functioning of PT Committee as the coordinator.
To oversee, the development of PT programs covering calibration and testing laboratories.
To oversee all work and activities in relation to the conduct of PT programs.
To create a web-site, to disseminate the information about PT Schedules.
To facilitate interested laboratories, so as to participate in forthcoming programs if interested.
Maintains the records of the completed PT programs
Comply with the requirements given in- ISO/IEC Guide 43, volume I and II, to manage the PT activity.

ISO/IEC Guide 43Part-1 "Development and operation of proficiency testing scheme"
ISO/IEC Guide 43 Part-2 "Selection and use of proficiency testing scheme by laboratory accreditation bodies"

8.Organization and design of PT scheme

Proper design of PT program is a must to ensure its success. The coordinator and the experts need to develop a plan and should include the following:

the name and address of the organization conducting PT program.
the name and address of the coordinator
nature and purpose of program/ nature of the test item/ test to be performed
procedure for selection of participants

the expected initial and target dates /deadlines of PT Scheme. Date for submission of results of testing.

9. Selection and preparation of appropriate test samples or measurement artefacts (Test item preparation)

test item/material to be distributed should be similar in type to those routinely tested.
the assigned value should not be disclosed to the participants until after the result have been collated.

procedures for sampling, randomizing, transporting, sorting and handling of the test items or materials to be documented.

bulk material prepared for PT must be sufficiently homogenous for each test parameter to ensure laboratories receive similar material.

10. Sample stability and transportability considerations

PT provider shall use a statistically random selection of a representative number of samples from a batch of test material to assess the homogeneity of the material. And the PT provider shall have a documented assessment procedure. Assessment shall be conducted in accordance to statistical design. In case of measurement artefacts, preliminary stability checks shall be made and periodic checks of assigned property values is carried out through out the course of the scheme.

it should be ensured and demonstrated that test items are sufficiently stable and have not undergone any significant change throughout the conduct of proficiency test.

PT provider shall provide proper packaging to the extent necessary to ensure conformity with relevant regional, national or international safety and transport requirements.

The PT provider shall ensure that material labels are securely attached to the product packaging of individual units and are designed to remain legible and intact within the period of use in a proficiency test.

11. Testing/calibration/ data analysis

The coordinator shall ensure that the participating laboratories have used the standard process of testing/calibration for which the program has been designed.

the data received from laboratories must be entered and analyzed and reported back to participants.

12. Statistical methods for treatment of PT data

The statistical technique used to analyze, the results need to be appropriate for each situation. These are three steps:

determination of assigned value
calculation of performance statistics
evaluation of performance

12.1 Determination of assigned values and its uncertainty

There are many methods for the establishment of assigned values. The most common procedures are listed below:

- a. Known values- with results determined by specific test item formulation (manufacture or dilution)
- b. Certified reference values- as determined by definitive methods (for quantitative tests)
- c.. Reference values- as determined by analysis, measurement or comparison of the test item alongside a reference material or standard, traceable to national or international standard.
- d.. Consensus value from expert laboratories - expert laboratories should have demonstrable competence in the determination of the measurand (s) under test, using validated methods known to be highly precise and accurate, and comparable to methods in general use.

12.2 Assigned values are determined to evaluate participants fairly.

12.3 The following statistics are used when assigned values are determined by consensus technique:

a. Qualitative value - consensus of a predetermined majority percentage (usually expressed on a nominal or ordinal scale)

b Quantitative value - "average" for an appropriate comparison group such as:

mean, which may be weighted or transformed (e.g. geometric mean, robust mean)
median, mode or other robust measure.

Where appropriate, the uncertainty of the assigned values should be determined using procedures described in Guide to the expression of uncertainty in measurement.

12.4 Method to deal with extreme results

When participants results are used to determine the assigned values, it is done by using robust statistical methods or by removing outliers prior to calculations (ISO 5725- 2). In larger schemes, it may be possible to have automated outliers screens.

a If results are removed as outliers, they are removed only for calculation of summary statistics. These results are still to be evaluated with in the proficiency scheme and given an an appropriate performance rating.

NOTE: 1. When assigned values are determined by reference or participant consensus, the coordinator should have a procedure to establish the trueness of the assigned values and for reviewing the distribution of the data.

NOTE: 2. The coordinator should have the criteria for the acceptability of an assigned value in terms of its uncertainty.

NOTE: 3. Organizing a PT or the inter-laboratory comparison program always needs involvement of more than ten laboratories to facilitate performing statistical analysis of the data obtained from laboratories.

13. Calculation and evaluation of performance

The objective is to measure the deviation from the assigned value to compare with performance criteria. And it needs transforming proficiency test results to be into a performance statistic, so to be able to do comparison with defined goals. Variability measures are often used for calibration of performance statistics. Common examples of such variability measures for an appropriate comparison include:

Standard deviation 's' (SD)

Coefficient of variation (CV) or relative standard deviation (RSD)

Percentile, median absolute deviation or other robust measures

13.1 Qualitative results: No calculation is usually necessary.

13.2 Statistics used for quantitative results is listed below

Difference ($x - X$), where "x" is participating result and X is the assigned value

Percentage(%) difference $(x - X) \times 100 / X$

Percentile or rank

13.2.1 Z scores and evaluating performance for Z Scores (inter-lab testing scheme)
where

$$Z = \frac{x - X}{s}$$

And x : participant results

X Assigned value (or mean)

' s ' an appropriate estimate/measure of variability which is selected to meet the requirements of the scheme. (e.g. standard deviation). This model can be used both in the situation where X and s are derived from participants results or when X and s are not derived the participants results. [for example when assigned values and variability are specified.

$|z| \leq 2$: satisfactory

$2 > |z| < 3$: questionable

$|z| \geq 3$: unsatisfactory

13.2.2 En number (Measurement comparison scheme)

. A convenient & internationally accepted statistical method for analyzing test results

En numbers (typically used in measurement comparison schemes), where

$$En = \frac{x - X}{\sqrt{U^2_{kb} + U^2_{ref}}}$$

and U_{kb} is the uncertainty of a participants result and U_{ref} is the uncertainty of the reference laboratory's assigned value. For En numbers, evaluating performance

$|En| \leq 1$ = satisfactory

$|En| > 1$ = unsatisfactory

Assigned values should be determined to evaluate participants fairly. This is accomplished through selection on common comparison groups, wherever possible, and the use of common assigned values.

The following are the list of items presented in this section.

1. WHAT IS PROFICIENCY TESTING

2. WHY PROFICIENCY TESTING IS IMPORTANT

3. ESSENTIAL REQUIREMENTS

4. HOW TO SOURCE A PROFICIENCY TESTING PROGRAMME

5. PROFICIENCY TESTING IN A DEVELOPING ACCREDITAION BODY

1. WHAT IS PROFICIENCY TESTING

Proficiency Testing is defined as a method of checking lab performance by means of inter-laboratory tests. It includes comparisons lab's results at intervals with those of other labs, with the main object being the establishment of trueness. It takes many forms and the design, management and operation of a number of these are described in ISO/IEC Guide 43. They range from one-on-one comparison of a laboratory's output with that of a

reference laboratory to extensive programmes involving many laboratories, often operating in many countries.

Some programs involve proficiency testing only with no on-site assessment or verification. These involve frequent programs in which all laboratories must participate and produce data acceptable to the program manager every time or with very few outlying results. This is not regarded as laboratory accreditation and is only applicable to the needs of the program operator with such proficiency testing regimes almost always focusing on a few tests critical to the needs of the program manager. Laboratory accreditation bodies may, however, use results from such programs as evidence of satisfactory performance in that area of testing.

Setting up of proficiency testing by bodies in developing countries is often very difficult. There may be too few laboratories in each particular field of testing to yield statistically meaningful results, or reference materials and reference materials and reference laboratories are not readily accessible and local legal requirements and administrative practices may inhibit easy access to proficiency testing programs operated by foreign organizations.

Nevertheless, for full international recognition, some proficiency testing activity must be included in any laboratory accreditation activity. Well operated programs demonstrate a degree of sophistication on the part of the accreditation body and successful participation in such programs adds credibility to the laboratory's claim for recognition of competence.

2. WHY PROFICIENCY TESTING IS IMPORTANT

To ensure that lab data are meaningful.

Helps in achieving mutual recognition and resolve disputes

Consistent good performance enhances reputation of laboratory.

Helps to identify and initiate corrective and preventive actions

Proficiency testing is used by laboratory accreditation bodies to complement the on-site peer assessment process and to provide objective data about the performance of individual laboratories. Proficiency testing may also be used for other purposes such as evaluation and validation of test methods, but, in accreditation, its primary role is to assess performance.

Basically, there are two main types of programs; concurrent testing of samples by groups of laboratories and programs involving the examination of a single item by laboratories in sequence. The former is most commonly used in conventional testing programs and the latter for calibration programs.

In the more mature accreditation bodies, where proficiency testing programs are a routine part of their operations, all laboratories are required to participate in regular programs relevant to their scopes of accreditation. Even then, it must be recognized that suitable programs are not always readily available for every area of testing.

In the large, technically sophisticated economies, a wide range of proficiency testing programs are available from commercial sources and accreditation bodies in those countries make use of those services. In smaller economies, there are fewer commercial services available and the accreditation body may be forced to provide appropriate proficiency testing programs; at least filling gaps left by the commercial providers.

The increasing insistence by accreditation bodies that accredited laboratories participate in proficiency testing programs operated by a variety of service providers has forced thinking towards accreditation of those services. ILAC has published a guideline document (ILAC G13) and some accreditation bodies have moved to offer accreditation, e.g. UKAS, but the topic remains under discussion. It is likely that, in the near future service providers, including accreditation bodies themselves, will need to seek some form of accreditation for that activity if the programs are to be recognized within the context of mutual recognition activity.

In the meantime, however, accreditation bodies are obliging their accredited laboratories to participate in proficiency testing programs from “reputable” sources and using the data generated as part of the information needed to make decisions regarding accreditation.

3. ESSENTIAL REQUIREMENTS

ISO/IEC Guide 43 spells out the requirements for development and operation of proficiency testing programs and criteria for their selection and use by accreditation bodies. In designing a particular program, care must be taken to ensure both technical validity and value in the end result. Right at the beginning, technical advice should be sought on at least the following issues:

Selection of tests to be conducted, the range of values to be covered, test methods to be used and samples to be prepared.

The paperwork to be prepared – instructions, result sheets, reporting requirements and formats and units of measurement

As in any testing activity, the value of the output is fundamentally dependent on the integrity of the samples to be tested. In a program involving a number of laboratories testing samples drawn from a bulk supply, this must be carefully prepared to ensure homogeneity and tested to confirm homogeneity. Samples must also be very stable otherwise there will, inevitably, be problems with transportation and later disputes over results.

Where a single or artefact is to be tested or measured sequentially by a number of laboratories it must be selected bearing in mind its ease of transportation, stability and robustness.

In any case, the sample or artefact must be well characterized prior to the commencement of the program.

ORGANIZATION OF PROFICIENCY TESTING PROGRAM

. Prepare whole of bulk material in a satisfactory form

Divide the material into required portions and pack in suitable, compatible containers.

Select about 10 – 20 containers randomly.

Homogenize contents of each selected container

From each container, take 2 test portions.

Analyze portions in random order.

Estimate sampling variance (including outliers)

Assign a precision standard at Mean analyte value

Homogeneous samples prepared by the organizers

Schedules prepared in advance, circulated and samples distributed.

Participants analyze samples and report results to the organizer.

Results analyzed and performance assessed.

Protocol of PT program :

Following Journals are references

The International Harmonized Protocol for the proficiency testing of (chemical) Analytical Laboratories.

J. AOAC International, 1993, 76, 926-940 and Pure and applied chemistry 1993, 65, 2123-2144

4. HOW TO SOURCE PROFICIENCY TESTING PROGRAMME

Even a preliminary search of the Internet yields well over one thousand providers of proficiency testing programs. Many of these are highly focused programs only available to a particular segment of a particular market, but a number offer services for a wide range of tests to laboratories anywhere in the world on a commercial basis.

On a less commercial scale, the regional accreditation cooperations, APLAC and EA, provide regular programs for both testing and calibration and participation from laboratories outside of those regions can be negotiated. These programs, however, are intended for a different purpose in that they attempt to compare performance of laboratories in one member country with laboratories in another or of laboratories in one region with those in the other. These regional and inter-regional programs permit only participation by a relatively few laboratories as they are looking for regional anomalies.

The model is that all accredited laboratories in a particular country participate in national schemes. Some of those then participate in regional schemes and fewer still participate in the international programs. A satisfactory result at all three levels enhances the credibility of the total network.

Nevertheless, some national accreditation bodies and the regional cooperation bodies offer their proficiency testing knowledge and skills to others so that a truly global system can be developed and the various mutual recognition arrangements strengthened. To this end, training courses are available and opportunities exist for staff in emerging bodies to be attached to more mature organizations to obtain extensive hands-on experience.

There are several P.T providers throughout the globe, some are listed below .
However, laboratories have to select a program and provider relevant to their purpose.

Links:

1. APLAC members : P.T. Schedule.

Link :

<http://www.aplac.org/proficiencytesting/schedule.htm>

2. A2LA members : P.T .Program details.

Link :

<http://www.a2la.org>

<http://www.aoac.org/>

<http://www.astm.org/STATQA>

<http://www.csl.gov.uk/>

<http://www.fapas.com/>

<http://www.collaborativetesting.com/>

[http:// www.proficiency.org/](http://www.proficiency.org/)

Cross Border Issues:

In the participation into a P.T program, the transportation of sample, maintaining the stipulated environmental conditions, custom clearance, quarantine issues and meeting the time schedule are very important.

Before issuing an order to a P.T provider, the Quality Manager has to look into these aspects.

An agency which can take-up the transportation job by maintaining the requisite environmental conditions (so that the integrity of sample is retained throughout the transportation until it reaches the laboratory) has to be identified .

Local applicable customs rules are to be thoroughly understood and the sample import shall be within the rules.

Quarantine issues are to be resolved . Some countries may not allow samples containing positive strains of microbes, toxins / pests etc. The Quality Manager has to inform the P.T provider before hand, to enclose necessary certificates along with the samples. Local permissions must be obtained in advance for smooth customs clearance.

5. PROFICIENCY TESTING IN A DEVELOPING ACCREDITATION BODY

Proficiency testing programs are expensive to operate and therefore great care is required to select areas of testing that have some importance either in the context of trade or for the domestic role of the accreditation body. Emerging bodies also face the reality of having very few laboratories in any one area of testing which increases the problem of being able to deal with the proficiency testing results in a statistically satisfactory way.

Importing samples from foreign programs is not always easy as local customs officials will be quite unfamiliar with the needs of the accreditation body and the laboratories. In many cases suitable samples may be prohibited from air transport by regulations (e.g flammable liquids, mercury in glass instruments) or the samples are too unstable for reliable transportation (e.g.fresh foods) . Never the less, an accreditation body seeking international recognition will need to address, to the best of its ability, the problem of having objective evidence of performance of its accredited laboratories.

In many cases, certified reference materials may be used. This is an expensive option and not without problems, but it may be the only alternative in some situations. One obvious problem occurs when the reference material is from a well known source and may be readily identifiable by the participating laboratories.

6.Reference Materials (CRM)

1.Introduction

Reference materials are substances with sufficient well-established properties, which are used for calibration of equipment, assessing measurement methods, chemical analysis and materials testing. The chemical measurement system that has evolved over the years involves production, purification and characterization of chemical substances as reference materials. These may be classified as Primary Reference Materials, Certified Reference Materials and Reference Materials depending on certain established norms. Institutions such as NIST of USA, EURALAB, BAM of Germany and the Laboratory of the Government Chemist, U.K. etc., are major providers of reference materials.

2.Certified reference Materials (CRMs)

Reference materials, accompanied by a certificate, showing that one or more property values are certified by a procedure, which establishes their traceability to an accurate realization of the unit in which the property values are expressed, Each certified value is accompanied by an uncertainty at stated level of confidence.

3.Reference Materials (RMs)

Materials or substances, one or more of whose property values are sufficiently homogeneous and well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

4.Calibration

Calibration is a set of operations that establish, under specified conditions, the relationship between the values of quantities indicated by a measurement instrument or measuring system or values represented by a material measure or a reference material, and the corresponding values realized by standards.

5.ISO/IEC 17025 & CRMs& RMs

Clause 5.6.1 " All equipment used for tests /or calibrations, including equipment for subsidiary measurements (e.g. for environmental conditions) having significant effect on the accuracy or validity of the results of the test, calibration or sampling shall be calibrated before being put into service."

Cause 5.6.2.1.2 "There are certain calibrations that currently, cannot be strictly made in SI Units, in these cases calibration shall provide confidence in measurements by

establishing traceability to appropriate measurement standards such as: - the use of certified materials, provided by a competent supplier to give a reliable physical or chemical characterization of a material."

6. The International Standard / documents on CRMs and RMs

- ISO REMCO, the international committees provide the relevant information.
- ISO Guide 30: 1992 Terms and definitions used in connection with reference materials
- ISO Guide 31: 1996 Contents of the certificates of reference materials certificates of reference materials is are documents that contain summarized descriptions of all information on the reference material. This guide shows all the items to be written in the table of contents of the certificate of reference materials.

Identity of producer and identification of the reference material

A description of the material and its intended use

Instructions on the correct use of the material

The assigned property values and the method used to derive these values

The date of certification and the period of validity of the certificate

Safety instructions

An indication of the level of homogeneity of the material

Signature and the name of the certifying officer(s) signing an analysis certificate; legal consideration

In the case of certified reference materials, the traceability and a statement of the uncertainty interval at a stated level of confidence must also be provided to clients.

- ISO Guide 32: 1997 "Calibration of chemical analysis and use of certified reference materials"

This guide contains general recommendations for those who are involved in quality assurance of testing and/or calibration laboratories,

- ISO Guide 33: 2000(E) "Uses of certified reference materials" In this guide, various terms concerning statistics are defined to express the uncertainty of the result of measurement or analysis in the form of confidence in the certified value.

ISO Guide 34: 2000 "Quality system requirements for reference material producers"

Since confidence in chemical measurements depends on the confidence in the reference material used for the measurements, it is necessary to assure the reference material. The basic policy of this guide is not to prove the quality of each reference material, but to assure the quality of the reference material by proving the scientific and technical

competence of the producer of the material. It contains the requirements for the producers of reference materials.

- ISO Guide 35: 2006 "Certification of reference materials-General and statistical principles"

Describes the role of reference materials in measurement science, measurement uncertainty, homogeneity of materials, general principles of certification, certification by definitive methods, certification by inter-laboratory testing, certification based on the metrological approach. ISO REMCO Document N 330.

"Contains a list of producers of certified reference materials, Information by task force 3 "promotion"

7.Types of reference materials

Pure substances characterized for chemical purity and/or trace impurities.

Standard solutions and gas mixtures, often prepared gravimetrically from pure substances and used for calibration purposes.

Matrix reference materials, characterized for the composition of specified major, minor trace chemical constituents. Such materials may be prepared from matrices containing the components of interest, or by preparing synthetic mixtures

Physico-chemical reference materials characterized for properties such as melting point, viscosity, and optical density.

Reference objects or artefacts characterized for functional properties such as taste, odour, octane number, flash point and hardness. This type also includes microscopy specimens characterized for properties ranging from fibre type to microbiological speciation.

8.Classes of Reference Materials

Primary reference material

Secondary reference Materials

In-house Working reference material

If the materials are available from other sources it may not be cost effective for laboratories do make working standards of their own. However when this is necessary , the guidance given in (i) ILAC requirement for the competence of reference material producers & (ii) X R Pan , Metrologia, 34, 35-39, 1997 must be complied with by non specialist laboratories, to ensure acceptance by accreditation bodies.A few of the issues that need to be addressed are: selection of materials (appropriateness, native materials versus spikes, material preparation etc.), homogeneity testing, preparation and packaging(homogeneity, contamination, stability etc.) , stability testing, certification studies,

uncertainty estimation, documentation and QA, certification approval, storage and distribution.

9. Traceability of reference materials

The use of appropriate reference material can provide essential traceability for chemical measurements and enable analysts to demonstrate the accuracy of results, satisfactory calibration of equipment and methods, enable monitoring of laboratory performance and validity of methods.

Two types of materials are recognized by ISO REMCO, (i) Certified reference materials (CRMs) and (ii) Reference materials (RMs).

CRMs, must by definition be traceable to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. Compared to CRMs, RMs are of lesser Quality and by definition are materials whose property values are sufficiently homogeneous and well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

10. Uses/role of CRMs and RMs

Reference materials/CRMs are used for following five purposes by the laboratories.

To calibrate measurement systems/analytical instruments

CRMs are used for method validation, development and evaluation

CRMs are used in quality assurance programs.

Laboratories also use reference materials for the quality assurance of analytical measurements. When unacceptable discrepancies arise in sample results between analysts using different methodologies, or the same method, the use of reference material can help identify and resolve the analytical problem. If analytical results are to be meaningful, the laboratory must adhere to a defined quality assurance program. Quality assurance in this context will be the adherence to a set of procedures meant to ensure validity of analytical results for intended purpose.

CRMs are used in PT program/inter laboratory comparison or round robin programs

CRMs for development of in-house standards

Reference materials that are produced by laboratories (in-house) are generally used on day to day basis. They can be verified by analysis alongside CRMs. The attraction of using in house standards is that they provide a relatively cheap option as compared to using certified reference material. It must be remembered that in-house reference materials do not replace CRMs, but only enable CRMs to be used on less frequent basis.

11. Selection of Reference material

In selecting a reference material for a particular application, it is the responsibility of the analyst to assess its suitability. As such, it is necessary to consider all or some of the following factors before selecting a material:

Matrix match and potential interferences

Measurands (analytes)

Measurement range concentration

Measurement uncertainty

Certification procedure used by the producer

Documentation supplied with the material (certificate report etc.)

Reference:

ISO Guide 31.

Use of reference materials in laboratories, by John Eames, NATA NEWS Volume 94.

The selection and use of reference material by Bernard King, NATA NEWS VOLUME 95.

Reference materials are one of the important tools in the realization of several aspects of Quality measurements. These also find their utility in :

1. Calibration of equipment
2. Method Validation
3. Estimation of uncertainty of measurement
4. Quality control
5. Quality assurance and
6. Training

The validity of measurements can be assured if, validated methods and equipments are used, which in turn depend on reference materials.

Further RMs are important in ensuring

1. Comparability with measurements made in other laboratories (traceability and measurement uncertainty)
2. Independent evidence of performance (proficiency testing)
3. Those of well defined QC and QA procedures
4. Qualified and competent staff undertake the work

In many cases, a single measurement operation serves more than one Quality purpose. To serve different functions, different types of reference materials are required. Method validation needs a certified reference material whereas a QC operation needs a working level reference material. The degree of purity, traceability and trueness determines their utility.

Reference Material (RM)

A material or substance once or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

Certified Reference Material (CRM)

A Reference Material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Some times reference materials are also classified as:

Primary reference material

Secondary reference material

In-house or working reference material

The uncertainty component decreases when moved from bottom to top

Reference Materials – Different types

Reference materials support measurements concerned with chemical composition, and quantification, physical, clinical, biological and engineering properties. These may be characterized for “Identity” or for “Property values”

Reference materials may be classified broadly into the following categories:

1. Pure substances characterized for chemical purity and/or trace impurities.
2. Standard solutions and gas mixtures, often prepared gravimetrically from pure substances and used for calibration purposes.
3. Matrix reference materials, characterized for the composition of specified major, minor or trace chemical constituents. Such materials may be prepared from matrices containing the components of interest, or by preparing synthetic mixtures
4. Physical-chemical reference materials characterized for properties such as melting point, viscosity, and optical density.
5. Reference objects or artefacts characterized for functional properties such as taste, odor, octane number, flash point and hardness. This type also includes microscopy specimens characterized for properties ranging from fiber type to microbiological specimens

Reference Materials – Traceability

Reference materials are important tools for the transfer of measurement accuracy between laboratories and their property values should, where feasible, be traceable to SI. Traceability is, however, a relatively new concept in the field of chemical measurement and as a consequence very few chemical reference materials are explicitly traceable to the relevant SI unit which is the mole. A hierarchy of methods is, however, used for assigning property values to materials and even if not stated; their traceability can be described as follows:

Measurement Method	Traceability
Primary method	SI
Method of known bias	SI / International standard
Independent method(s)	Results of specified methods
Inter-laboratory comparison	Results of specified methods

A combination of certification procedures is sometimes employed, such as consensus value derived from an inter-laboratory comparison where primary methods were used. In the absence of formally stated traceability it will be necessary for the user to make judgments about implicit traceability, based on the certification data available in reports and technical literature. It is important to be aware that chemical interferences and matrix bias are not uncommon and contribute to lack of agreement on measurements.

The measurement uncertainty of the property value of a reference material employed in a measurement process will contribute to the uncertainty of the final measurement but should contribute less than one third of the overall measurement uncertainty. Any underestimation of the uncertainty of the RM property value will, of course, be carried through to measurements where the RM is used.

Reference Materials – Selection and availability

Generally the demand for reference materials exceeds supply in terms of range of materials and availability. It is rare to have a choice of alternative RMs and the user must choose the most suitable material available. It is important therefore that users and accreditation bodies understand any limitations of reference materials employed.

There are, however, several hundred organizations producing tens of thousands of reference materials worldwide. Producers include internationally renowned institutions such as NIST; collaborative government sponsored programs such as the EU BCR program, semi-commercial organizations. The distinction between government institutes and commercial businesses is disappearing with the privatization of a number of national laboratories.

Not all materials that are used as reference materials are described as such, commercially available chemicals of varying purity, commercial matrix materials and products from research programs are often used as standards or reference materials. In the absence of certification data provided by the supplier it is the responsibility of the user to assess the information available and undertake further characterization as appropriate. Guidance on the preparation of reference materials is given in ISO Guides 31, 34 and 35 and guides on the preparation of working level reference materials are also available..

Information about reference materials is available from a number of sources. The COMAR Database, contains information on more than 10,000 RM's / CRM's which can be accessed directly through institutes which provide an advisory service. Further information can be obtained from the COMAR Central Secretariat (comar@BAM.de). Advisory services assist users to identify the type of material required for their task and to find a supplier. A database covering reference materials currently under development has been prepared by CITAC and ISO REMCO (14). A number of suppliers provide a comprehensive range of materials including materials produced by other organizations, and aim to provide a one-stop-shop for users. A first internet database for a selection of RMs has recently appeared and is likely to be followed by others (<http://www/iaea/org/programmes/nahunet/e4/nmrm/index.htm>) provided by some RM providers.

Reference Materials – USES

a) Measurement uncertainty and Method Validation

Estimation of bias (the difference between the measured value and the true value) is one of the most difficult elements of method validation, but appropriate RMs can provide valuable information, within the limits of the uncertainty of the RMs certified value(s) and the uncertainty of the method being validated. Although traceable certified values are highly desirable, the estimation of bias differences between two or more methods can be established by use of less rigorously certified RMs. Clearly the RMs must be within the scope of the method in terms of matrix type, analytical concentration etc., and ideally a number of RMs covering the full range of the method should be tested. Less rigorous bias studies can be employed where minor modifications to a well-established method are being evaluated.

Replicate measurement of RM, covering the full range of variables permitted by the method being validated can be used to estimate the uncertainty associated with any bias, which should normally be corrected for.

The uncertainty associated with a RM should be no greater than one third of that of the sample measurement.

b) Verification of the correct use of Method

The successful application of a valid method depends on its correct use, both with regard to operator skill and suitability of equipment, reagents and standards. RMs can be used

for training, for checking infrequently used methods and for trouble shooting when unexpected results are obtained.

c) Quality Assurance and Quality Control

QA and QC programs are best performed by using RMs. These can be introduced as an unknown sample amongst similar samples. The result obtained for the RM guides the authenticity of the procedure / process followed for the subject batch.

Assessment of suitability of RMs

As previously indicated the key quality parameter is the uncertainty associated with the certified value and the reliability of the uncertainty estimate. Uncertainty budgets should be derived using the ISO approach. Certification data should be stated together with the expanded uncertainty, U , using a coverage factor $k=2$.

However, the full uncertainty data is often not available and it is necessary to consider other quality criteria. Also, the non-expert may not be in a position to fully evaluate the certification data and quality check list, or a third party quality approval system, is desirable. Such systems are under development but may take some time to become fully established.

A protocol for assessing the suitability of RMs is detailed in Figure 2 and discussed below. The user must assess the appropriateness and fitness for purpose of any RM based on the customer and analytical requirements. Factors to be considered include the following:

1. The suitability of a reference material depends on the details of the analytical specification. Matrix effects and other factors such as concentration range can be more important than the uncertainty of the certified value as detailed in (11). The factors to consider include:

Measured parameters.

Measurement range (concentration)

Matrix match and potential interferences

Sample Size

Homogeneity and stability

Measurement uncertainty

Value assignment procedures (measurement and statistical)

2. The validity of the certification and uncertainty data, including conformance of key procedures with ISO Guide 35 and other ISO requirements.

3. Track record of both the producer and the material. For example, when a RM in use has been subjected to an inter-laboratory comparison, cross-checked by use of different methods, or there is experience of use in a number of laboratories over a period of years.

4. Availability of a certificate and report conforming to ISO Guide 31.

5. Demonstrated conformance of the production of the reference materials with quality standards such as ISO Guide 34 or ILAC requirements, or compliance of the measurement of property values with ISO/IEC requirements.

All or some of the requirements may be specified in the customer and analytical specification, but often it will be necessary for the analyst to use professional judgment. Finally, quality does not necessarily equate to small uncertainty and fitness for purpose criteria need to be used.

Laboratories must be able to explain and justify the basis of selection of all RMs and of course any decision not to use a RM. In the absence of specific information it is not possible to assess the quality of a RM. The rigor with which an assessment needs to be conducted depends on the criticality of the measurement, the level of the technical requirements and the expected influence of the particular RM on the validity of the measurement. A formal suitability assessment is required only where the choice of RM can be expected to significantly affect measurement results.

Reports and Certificates

Ideally, a certificate complying with ISO Guide 31 and a report covering the characterization, certification and statistical analysis procedures, complying with ISO Guide 35, will be available. However, many RMs, particularly older materials and materials not specifically produced as RMs, may not fully comply with ISO Guides 31 and 35. Alternatively, equivalent information, in whatever form it is available, that provides credible evidence of compliance can be considered acceptable. Examples include the following: technical reports, trade specifications, papers in journals or reports of scientific meetings and correspondence with suppliers.

In-House preparation of Reference Materially

High quality RMs is demanding and costly to produce and if materials are available from other sources it is not normally cost effective for laboratories to make their own. However should this be necessary, there are guides available to help the non-specialist laboratory prepare their own RMs. Some of the key issues that need to be considered are: selection of materials (appropriateness, native material versus spikes, material preparation etc.), homogeneity testing, preparation and packaging (homogeneity, contamination, stability etc.), stability testing, certification studies, uncertainty estimation, documentation and QA, certification approval, storage, distribution and lifetime re-evaluation.

7. Test Method

Introduction

The requirement of ISO/IEC 17025 is that the test/calibration method has to be from standard written instructions. However, the standard (ISO/IEC 17025) allows flexibility in adopting a non-standard test/calibration method , subject to certain conditions and where required subject to validation. It is also flexible in accepting a test/calibration method that has been developed by the laboratory but has been subjected to full validation. It also permits use of Industry standard methods.

It is the responsibility of the laboratory To provide a method which is fit for purpose. This may involve discussions with the customer. In the end the customer can specify the method but under these circumstances the laboratory has the responsibility to inform the customer if the method being insisted upon is not ideally suited to the purpose the customer requires.

The following are the possible situations where the laboratory is required to make its own judgment to select a test method:

- Client's wants and needs
- Regulatory requirements
- Industry acceptance
- Sampling and sample preparation
- Environmental requirements
- Staff capability requirements
- Repeatability, reproducibility and uncertainty
- Recovery and matrix effects
- Safety
- Cost and time

The introduction of test and calibration methods developed and validated by the laboratory shall be a planned activity and shall be assigned to qualified personnel, equipped with adequate resources. Plans shall be updated as development proceeds and effective communication amongst all is ensured.

Validation is the confirmation by examination, by established techniques, with the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Each test/calibration method has its own uncertainty of measurement and this standard requires determination of this value.

The user of the test methods should be in position to evaluate its performance on the basis of:

Matrix effect

Linear concentration range, accuracy and repeatability

Justification for deviation from standard methods

Scientific basis /logic for deviation from standard procedure

Identification of key or critical steps

In-house quality control

Test Methods

Labs shall use appropriate methods and procedures for all tests and / or calibrations within its scope. These include sampling, handling, transport, storage and preparation of items to be tested and / or calibrated. The laboratory shall have instructions on the use and operation of all relevant equipment and on the handling and preparation of items for testing or calibration or both where the absence of such instructions could jeopardize the results of tests or calibration. Deviation from test and calibration methods shall occur only if the deviation is documented, technically justified, authorized and accepted by the customer.

- A. Selection of test methods
- B. Laboratory developed methods
- C. Non standard methods
- D. Sources of methods

A. Selection of test methods.

The laboratory shall use test and calibration methods including methods for sampling which meet the needs of the customer and which are appropriate for the tests or calibrations it undertakes. Methods published in international, regional or national standards shall be preferably used. The laboratory shall ensure that it uses the latest valid edition of a standard unless it is not appropriate or possible to do so. When necessary, the standard shall be supplemented with additional details to ensure consistent application. When the customer does not specify the method to be used, the laboratory shall select appropriate methods that have been published either in international , regional or national standards, or by reputable technical organizations, or in relevant scientific text or journals

or as specified by the manufacturer of the equipment. Laboratory developed methods or methods adopted by the laboratory may also be used if they are appropriate for intended use and if they are validated. The customer shall be informed as to the method chosen. The laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations. If the standard method changes, the confirmation shall be repeated.

B. Laboratory developed methods.

The introduction of test and calibration methods developed by the laboratory for its own use shall be a planned activity and shall be assigned to qualified personnel equipped with adequate resources.

C. Non standard methods.

When it is necessary to use methods not covered by standard methods, these shall be subject to agreement with the customer and shall include a clear specification of the customer's requirement and the purpose of the test or calibration. The method developed shall have been validated appropriately before use.

D. Sources of methods.

Test methods are specific for the intended use. Several countries have published their National methods. Some times methods of international origin have been adopted as their National methods. In general, product-wise test methods can be obtained from:

1. Test methods on water

a) U.S. Environmental Protection Agency

1. <http://www.epa.gov/waterscience/methods/>
<http://www.epa.gov/waterscience/pc/>

b) American Public health association:

<http://www.apha.org>

2. Solid waste management : National technical information service

Paid site

http://www.ntis.gov/products/families/environmental_test_methods.asp?loc=4-3-2

3. Foods and allied Products:

a) AOAC

<http://www.aoac.org>

b) CODEX Alimentarius

http://www.codexalimentarius.net/web/standard_list.do

c) US FDA

<http://www.cfsan.fda.gov>

4. Drugs and Pharma products

a) US Pharmacopoeia

<http://www.usp.org/USPNF/>

b) British Pharmacopoeia

<http://www.pharmacopoeia.co.uk/>

c) Indian Pharmacopoeia

<http://www.mohfw.nic.in/kk/95/ia/95ia0e01.htm>

e) Japanese Pharmacopoeia

<http://jpdb.nihs.go.jp/jp14e/>

5. Chemicals and other Products

American Society for Testing and Materials Standards.

<http://www.astm.com>

6. International Organization for Standardization:

<http://www.iso.org/cate/cat.html>

7. American Society of Materials

<http://www.asminternational.org>

8.Method Validation

1.Introduction

By definition method validation is the process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent. Which analytes can it determine, in which matrices in the presence of which interferences? Within these conditions what levels of precision and accuracy can be achieved?

A test or calibration Method validation, aims at providing an assurance of reliability of data. The reliability and comparability of data can be guaranteed only through the implementation of quality assurance system including the application of method validation according to international accepted procedures and performance criteria.

2.When should be a method validated?

The test or calibration methods that are being used by laboratories are normally from standards bodies or other professionally established international bodies like the Association of official Analytical Chemists (AOAC International).Even such standard methods will require some validation. Basically the laboratory will need to determine the accuracy and precision of the method in its hands as a minimum. Limit of detection may also need to be established if relevant, e.g. in environmental work.

However, when laboratories use test/calibration methods that are not from the above type of standard source full validation will be required.

3.Factors to be Considered in Method Validation

The minimum requirements of method validation are bias, precision and LOD discussed below. In the case of methods developed from scratch in-house a much more comprehensive approach covering the other parameters described below will be required. Few laboratories, however, take this approach and the norm is to adopt and perhaps slightly modify standard methods.

Precision: Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples.

The two most common precision measures are 'repeatability' and 'reproducibility'. These are expression of two extreme measure of precision which can be obtained. Repeatability

(the smallest expected precision) will give an idea of the sort of variability to be expected when a method is performed by a single analyst on one piece of equipment over a short time scale. If a sample is analyzed by a number of laboratories for comparative purposes then a more meaningful precision measure to use is reproducibility (this is the largest measure of precision).

In practice the laboratory is usually interested in the extent of variability which occurs over time when it operates the method. This is called 'intermediate precision' and describes the variability when the method is deployed in the same laboratory, perhaps on different pieces of equipment, and using different analysts on the staff. It is expected that this will give a value between repeatability and reproducibility.

Accuracy: Accuracy is popularly used to describe the measure of exactness of an analytical method, or the closeness of an agreement between the value, which is accepted as a conventional, true value or as an accepted reference value, and the value found. Accuracy is properly a qualitative concept and the correct term is 'bias'.

The bias of a method is an expression of how close the mean of a set of results (produced by the method) is to the true value. Bias is usually determined by study of relevant reference materials or by spiking studies.

Limit of detection: Limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be distinguished from a blank. It is expressed as a concentration at a certain specified signal-to-noise ratio, usually two-or three to one.

Where measurements are made at low analyte level e.g. in trace analysis, it is important to know what is the lowest concentration of the analyte that can be confidently detected by the method. The importance in determining this, and the problem associated with it arise, from the fact that the probability of detection suddenly changes from zero to unity as some threshold is crossed. For validation purposes, it is normally sufficient to provide an indication of the level at which detection becomes problematic.

Limit of quantification: The limit of quantification (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Like LOD, LOQ is expressed as a concentration, with the precision and accuracy of the measurement also reported. Often a signal to noise ratio of ten to one is used to determine LOQ. It is also known as 'limit' of determination.

The overall effect is that the laboratory will state that an analyte is detectable between LOD and LOQ but will not offer a result as the precision is unacceptable. Below LOD the analyte is not detected and above LOQ it is measured.

Selectivity/Specificity:

For analytical methods, the power of discrimination between the analyte and closely related substances (i.e. isomers, metabolites, degradation products endogenous substances, matrix constituents) is important

Therefore, potentially interfering substances must be chosen, and relevant blank sample must be analyzed, to detect the presence of possible interferences and to estimate the effect of the interferences.

In general, analytical methods can be said to consist of a measurement stage, which may or may be preceded by an isolation stage. It is necessary to establish that the signal produced at the measurement stage, which has been attributed to the analyte, is only due to the analyte and not from the presence of something chemically or physically similar or arising as coincidence. Whether or not other compounds interfere with the measurement of the analyte will depend on the effectiveness of the isolation stage and the Selectivity/Specificity of the measurement stage. Selectivity and Specificity are measures, which assess the reliability of measurement in the presence of interference. Specificity is generally considered to be 100% selectivity, but this agreement is not universal. Where the measurement stage is not specific, it is possible to state that certain analyte do not interfere.

Linearity and Range:

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line.

Range is the interval between lower and upper levels of analyte(inclusive) that have demonstrated to be determined with precision, accuracy and linearity using the methods as written.

Ruggedness: Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions, expressed as % RSD. These conditions include different laboratories, analysts, instruments, reagents, days etc.

Robustness: Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters.

4. Conclusion

A group of experts would have developed standard methods collaboratively. In theory this development should include consideration of all of the necessary aspects of validation. However, the responsibility remains with the user to ensure that the validation documentation is complete to the needs. Even if the validation is complete, user has to ensure that it is fit for purpose.

5. Validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose.

For pharmaceutical methods, guidelines from the United States Pharmacopeia (USP)

(1), International Conference on Harmonization (ICH)

(2), and the Food and Drug Administration (FDA)

(3, 4) provide a framework for performing such validations.

In general, methods for regulatory submission must include studies on specificity, linearity, accuracy, precision, range, detection limit, Quantitation limit, and robustness.

Method Validation for Chromatography

The purpose of this technical review guide is to present the issues to be considered when evaluating chromatographic test methods from a regulatory perspective.

The document discusses the points to note and weaknesses of chromatography so that CDER reviewers can ensure that the method's performance claims are properly evaluated, and that sufficient information is available for the field chemist to assess the method.

Analytical terms, as defined by the International Conference of Harmonization (ICH), 1993, have been incorporated in this guide.

Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids.

The components monitored include chiral or achiral drug, process impurities, residual solvents, excipients such as preservatives, degradation products, extractable and leachables from container and closure or manufacturing process, pesticide in drug product from plant origin, and metabolites.

Validation of chromatography methods

Link: <http://www.fda.gov/cder/guidance/cmc3.pdf>

Analytical Method Validations

The ability to provide timely, accurate, and reliable data is central to the role of analytical chemists and is especially true in the discovery, development, and manufacture of pharmaceuticals.

Analytical data are used to screen potential drug candidates, aid in the development of drug syntheses, support formulation studies, monitor the stability of bulk pharmaceuticals and formulated products, and test final products for release. The quality of analytical data is a key factor in the success of a drug development program.

The process of method development and validation has a direct impact on the quality of these data.

Reference

The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics (1998)

Above document can be down loaded from

<http://www.eurachem.org/>

and click on "guides and documents" tab

9.Uncertainty of Measurement

1.Introduction

Measurement is a process, in which a set of operations are performed to determine the value of quantity. A process is an integral set of activities that uses resources to transform inputs to outputs. In a measurement process even when all the measurement factors which can be controlled are controlled, repeated observation made during under the same condition, are rarely found identical. This is due to the variables - operator, reference standards / materials / Instrument / environment, calibration, test methods and therefore measurement results are never true value and in fact accompanied with uncertainty. Therefore the "measurement uncertainty" is a property of a measurement.

2.Essentials of measurement uncertainties

The customer needs to get an idea of the applicability of the result. Measurement uncertainty has to be taken into account particularly when regarding specification limits. Testing laboratories need uncertainties with their calibration certificates, so that they can establish the uncertainty of their own measurements.

An estimation of a measurement's uncertainty is required for testing and calibration laboratories complying with ISO/IEC 17025.

A calibration laboratory ... shall have and shall apply a procedure to estimate the uncertainty of measurement for all calibrations ...",

Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement"

The uncertainties values have to be stated in the test report depends on requirements by the test method, requirements by the customer, or whether conformance to specification has to be assessed (ISO/IEC 17025, 5.10.3).

In calibration, uncertainties have to be stated in the certificate (as they are required by the user of the calibrated equipment).

3. Scope

The purpose of this write-up is to familiarize the reader with subject of measurement uncertainty to that extent that he would be able estimate uncertainty in measurement in his laboratory.

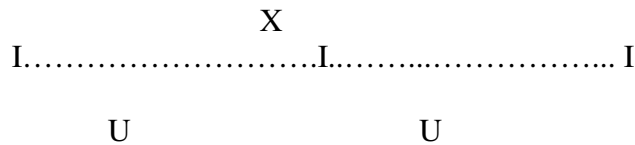
Instead of going into theoretical aspect and in great detail this document has taken the help simple and solved examples to familiarize and develop the skill of calculating uncertainty in measurements.

The examples given cover most of the situations but the focus is on chemical testing laboratory.

Hope, those who have very little or no exposure to the estimation of uncertainty will be able to start estimating uncertainty in measurement for their kind of lab.

4. Definition

Uncertainty is the parameter associated with the result of a measurement that characterizes the dispersion of the values that could be attributed to the measurand.



4.1 Uncertainty in Measurements: Defines a range that could be reasonably be attributed to the measurement result at a given confidence. Eg 56.2 ± 9.6 mg/L

Sources of measurement uncertainties in testing There are many possible sources of uncertainty, e.g. sampling, instrument drifts and calibration, homogenization and dilution effects, human factors, environmental effects

5. ISO GUM Principles I

- Specify what is being measured
- Identify what causes the result to change
- Quantify the uncertainty components

5.1 ISO GUM Principles II

Types of uncertainties

Type A: These uncertainties are evaluated by statistical analysis of a series of observations

Type B: These uncertainties are evaluated from any other information, such as information from past experience of the measurements, from calibration certificates, manufacturers specifications, etc.

Type A and B uncertainties are based upon probability distribution.

Type A uncertainties are estimated on the basis of repeat measurements, usually assuming the normal or "t" distribution for the variability in the mean of the values.

Type B uncertainty, by and large are obtained by assuming a particular probability distribution, such as normal, a rectangular or a triangular distribution.

5.2 ISO GUM Principles III

Convert to standard uncertainties

Combining the uncertainties U_c
Express as expanded uncertainty, U_c ,

Expanded uncertainty $U_c = k \cdot U_c$.
Where k is 'coverage factor'

6.Measurement Uncertainty Estimation Protocol

Conditions of the quality assurance data are comparable with the treated problem, e.g. matrix and composition, range of values, repeatability / reproducibility contributions the presumed simplifications are appropriate and acceptable .

7.Mathematics involved in estimating standard uncertainties

Let there be $X_1, X_2, X_3, \dots, X_n$ measurements

$$i. \text{ Mean } (\bar{x}) = \frac{X_1 + X_2 + X_3 + \dots + X_n}{n} = \frac{\sum X_i}{n}$$

ii standard deviation is given by

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

iii. Combining uncertainty/deviation

$$U = \sqrt{(U_i)^2 + (U_{ii})^2}$$

$$u(c)/C = \sqrt{(u(w)/w)^2 + (u(v)/v)^2 + (u(p)/p)^2}$$

iv. Expanded uncertainty U_c

$$U_c = k \cdot U_c, \text{ Where } k \text{ is 'coverage factor'}$$

7.1 Example of Type A evaluation: Type A method of evaluation of standard uncertainty applies to those situations where several independent observations have been made for any of the input quantities under same condition of measurement. If there is sufficient resolution in the measurement process, there will be an observable scatter or spread of the values obtained. These uncertainties are evaluated by the statistical analysis of a series of repeat measurements.

Case study -1: Procedure for estimation of type 'A' measurement uncertainty, based on internal reproducibility standard deviation.

Given data: These are ten results measured at different times, using different analysts.

(In reality laboratory staff is required to generate such information, for a specific situation to estimate standard uncertainty. This standard uncertainty, is used for estimating expanded uncertainty for an individual measurements).

Number of Determinations	Date of measurement	Result mg/Kg x_i	Result mg/Kg $x - \bar{x}$	Result mg/Kg $(x_i - \bar{x})^2$
1	4.6.07	100.0	-2.4	5.76
2	5.6.07	103.9	+1.5	2.25
3	6.6.07	104.8	+2.4	5.76
4	7.6.07	104.0	+1.6	2.56
5	10.6.07	101.9	-0.5	0.25
6	11.6.07	103.0	+0.6	0.36
7	13.6.07	103.8	+1.4	1.96
8	14.6.07	99.5	-2.9	8.41
9	17.6.07	100.2	-2.2	4.84
10	18.6.07	102.9	+0.5	0.25

$$\text{Mean } (\bar{x}) = \frac{X_1 + X_2 + X_3 + \dots + X_n}{n} = \frac{\sum X_i}{n} \dots\dots\dots (1)$$

Where \bar{x} is the mean of ten readings, & its value is = 102.4

And the standard deviation/standard uncertainty is given by

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} = \dots\dots\dots (2)$$

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} = \sqrt{\frac{32.4}{10-1}} = 1.897$$

n is the number of determinations

(n-1) is degree of freedom

S internal reproducibility standard deviation or standard uncertainty

\bar{x} is the mean

Relative reproducibility standard deviation = RSD

is $RSD = S / \bar{x} \dots\dots\dots (3)$

$$1.897 \text{ RSD} = S / \bar{x} = \dots\dots\dots = 0.0185$$

102.4

The magnitude of the obtained internal reproducibility standard deviation should be evaluated in relation to the field of application of the method (fitness for purpose) Correspondingly, the measurement uncertainty may be estimated, using results of replicate determinations carried out on reference materials, or from results of studies where the method under study was used in parallel with a well defined, established

reference method. Calculations are carried out as above using the reference value in place of the mean.

8.Expression of measurement uncertainty (for results from chemical laboratory)

The measurement uncertainty is a parameter, which describes in a quantitative manner the variation to the concentration of an analyte present in the sample. For an estimated measurement uncertainty to be of value, it must be estimated and expressed in a standardized manner.

Measurement uncertainty expressed as the standard measurement uncertainty, is defined (i) as 'measurement uncertainty of the result as one standard deviation'. And expanded measurement uncertainty, is defined as ' a quantity which defines the interval about the result, including a large portion of the variation which would result from the analyte present in the sample, and which is obtained by multiplying the standard measurement uncertainty with a coverage factor to obtain an estimate of the confidence interval for a measurement at a specific level of confidence'. This coverage factor usually equals 2; in some cases 3 may be used. Application of coverage factor of 2 corresponds to confidence level of approximately 95% , and 3 to a confidence level of more than 99%. The expanded measurement uncertainty is given by:

i. U (expanded uncertainty) = 'K.' X. 'C' X. 'RSD' -- -(4) And

ii U (uncertainty) = $\sqrt{\sum (x_i - \bar{x})^2/n-1}$. X. 'K' -- -(4a)

Equation -(4a) is used to estimate standard uncertainty.

Where K is the Coverage factor,

RSD is the relative standard deviation,

and C is the concentration of analyte.

As per recommendations a Coverage factor '2' is used. (what is a coverage factor and why a value of ' 2' has been taken will be discussed later)

The calculated expanded measurement uncertainty " U" represents half of the measurement uncertainty interval. Measurement result $\pm U$

Example -1 (Internal reproducibility) The measured concentration of an analyte of a solution is 99.4 mg/Kg. Determine expanded uncertainty.

$U = 'K' .X 'C' X 'RSD'$ Equation-(4)

$U = 2 \times 99.4 \times 0.0185 \text{ mg/Kg} = 3.7 \text{ mg/Kg}$

The measured result 99.4 mg/Kg accompanied by its expanded measurement uncertainty Is given by as $99.4 \pm 3.7 \text{ mg/Kg}$ Every time and for each test (when concentration of the same analyte is measured) Equation -4 is used to determine the value of expanded Uncertainty. What is K (Coverage factor) and where from it has come?

By definition it is a numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty.

it signifies to what extent the value of probability is associated with a confidence interval or a statistical coverage interval.

Confidence Interval: the interval about the mean within which the true value is expected to lie with the specified level of confidence.

9. Probability distribution

Uncertainty can only be defined in terms of probability/ level of confidence.

Any measurement will have some uncertainty and the quoted interval will be the range within which the true value lies at a certain level of confidence.

The relationship between the standard deviation and the confidence interval actually depends on the type of distribution to which measurement result conforms.

9.1 Types of probability distribution

The normal distribution

The rectangular distribution

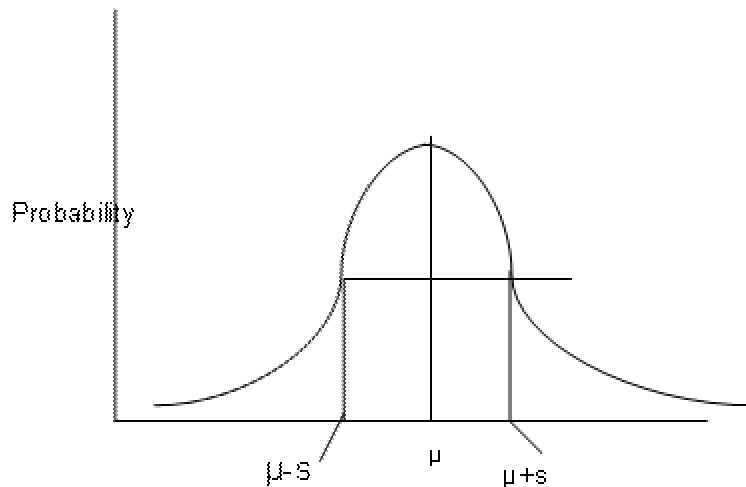
The triangular distribution

9.2 Normal distribution

The probability density function is given by =

$$P(x) = \frac{1}{s\sqrt{2\pi}} \cdot \exp\left[-\frac{(x-\mu)^2}{2s^2}\right] \quad -\infty < x < +\infty \quad (5)$$

Where " μ " is the mean and "s" is the standard deviation.



Normal distribution

The normal distribution has a maximum probability at the value, which is the mean of the measurements made, and falls away on both sides towards zero probability. It never reaches zero but approaches it exponentially since there is always a finite chance that a measurement will have any value between plus and minus infinite but further away from the mean you go, lesser is the probability.

The percentage values of the covered area of the curve are the confidence level, which is an indication of Coverage factor K, as per table -1

Table-1: Confidence level and the corresponding coverage factor (K)

confidence level	67.27%	90%	95%	95.45%	99%	99.73%
Coverage factor K	1.000	1.645	1.96	2.000	2.576	3.000

9.2.1 When to use normal distribution

- In some situations, the quoted uncertainty in an input or output quantity is stated along with level of confidence. In such cases, one has to find the value of coverage factor by using the Table-1,
- In the absence of any specific knowledge about the type of distribution, one may assume it to be normal distribution.
- When the uncertainty in a calibration certificate is given as a confidence interval/confidence level or in terms of a standard deviation multiplied by a coverage factor.

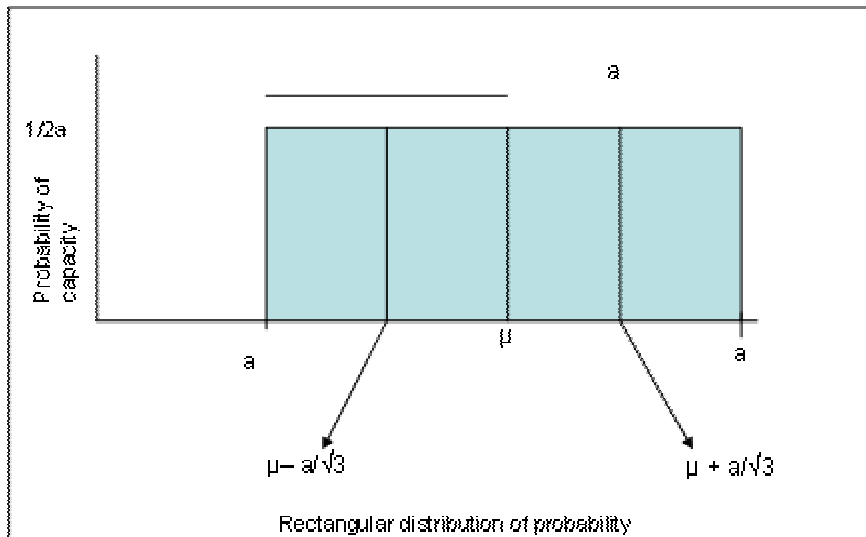
Like: When the calibration certificate shows calibration temp. 37°C with an uncertainty of $\pm 0.014^\circ\text{C}$ at 95% confidence interval. Uncertainty = $\sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$. X Coverage factor
 In such a situation one generally assume that the data has been subjected to full statistical analysis and is generally described by a normal or Gaussian probability distribution.

9.3 Rectangular distribution

The probability density function $p(x)$ of a rectangular distribution is as follows

$$P(x) = 1/2a, \quad a_- < x < a_+, \quad \text{where } a = (a_+ - a_-),$$

Given below is the figure that is representative for rectangular distribution.



9.3.1 When to use rectangular distribution ? In those cases, where it is possible to estimate only the upper and lower limits of an input quantity (X) and there is no specific knowledge about the concentration of values of (X) within the interval, one can only assume that it is equally probable for X to lie anywhere within this interval.

9.3.2 Illustration for rectangular distribution

i. Volume

5 cc class bulb pipette has tolerance of ± 0.03 c.c. Tolerance means that a particular pipette has a volume between 4.970 cc to 5.030 cc at 20°C. It is due to the control in manufacturing process, and most of the pipettes made are close to 5.00 cc, and only few are outliers.

But we can not say, in what way this volume, 5 c.c is distributed in all the pipettes manufactured and available in lab, but one can assume that true volume in the pipette of the lab has an equal probability of being any value in the range 4.970 cc to 5.030 c.c.

The probability distribution for the pipette volumes then must be assumed to be such that there is constant probability throughout the tolerance range, 4.97 cc to 5.030 cc, and zero probability outside this range.

The distribution shows that any capacity between 4.97 cc and 5.03 cc has equal probability and any capacity outside this range zero probability. The actual probability of any capacity within the range is $1/2a$, where a is the half width of the distribution. This is a simple requirement arising from the fact that the area under the distribution, which represents the total probability of all capacities, must be unity, i.e. $2a \times 1/2a$.

And for the rectangular distribution the standard deviation is given by $a/\sqrt{3}$

When dispersion of measurement is represented by a rectangular distribution, then the standard uncertainty is the half width of the distribution divided by the square root of three, i.e. $1/\sqrt{3}$.

Though the tolerance of a 5 cc class B pipette is ± 0.03 cc, the standard uncertainty in the 5.00 cc capacity pipette is $0.03/\sqrt{3} = 0.03/1.732 = 0.017$ cc.

Conclusion: When calibration data is given in terms of tolerance interval, then in evaluating its standard uncertainty, rectangular distribution is presumed.

ii. Volt meter (digital instruments)

Suppose in a lab there is a digital volt meter which reads to the nearest milli-volts and it is reading 5.000 volt. As the voltage applied increases we would expect the meter to change to reading 5.001 volts when the actual voltage passes 5.0005 volts. A similar situation will apply on a decreasing voltage. This means that when the meter reads exactly 5.000 volts the true voltage may be anywhere between 4.9995 volts to 5.0005 volts.

Since the meter is digital and provides no information across the interval between changes on the digital display we must assume that, when the meter reads 5.000 volts, the true voltage is equally likely to be anywhere in the interval of 4.9995 to 5.0005 volts.

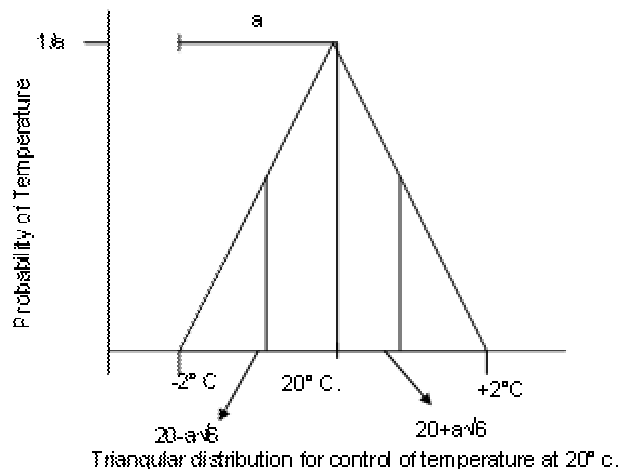
This means that the uncertainty in the reading, resulting from the resolution of the meter display is represented by a rectangular probability distribution of half width 0.5 millivolts so the uncertainty in the meter reading resulting from readability of the meter is $0.5/\sqrt{3} = \pm 0.29$ millivolts.

iii. Digital balance

The other commonest device in a laboratory with a digital scale is a balance. The principle is exactly the same as for voltmeter. For example a three place balance will read to the nearest milligram so the half width of the rectangular distribution is 0.5 milligram and the uncertainty due to the readability is $0.5/\sqrt{3} = \pm 0.29$ milligram.

The above examples give a general result for a digital device with a scale which registers in unit steps, the uncertainty resulting from the readability of the display is given by 0.29 in the units of the digital interval. And uncertainty due to readability of a four place balance is 0.29 tenths of a milligram, e.i. 0.029 milligrams.

9.4 The Triangular Distribution When the greatest concentration of the values is at the centre of the distribution, it is triangular distribution. For example when temperature is to be controlled $20 \pm 2^\circ\text{C}$, using thermostat which is calibrated, thermostat will hold the temperature very close to 20°C . This shown in given fig.



The distribution implied by this picture is that the most likely value for the temperature is 20°C with decreasing probability across the range 18°C to 22°C and zero probability outside this range.

The distribution shows the centre weighing of the temperature, with zero probability of the temperature being outside the ±2°C tolerance. The probability of a temperature of exactly 20°C is 1/a, where a is the half width of the distribution and the total probability is the area of the triangle, half of the base times the height, $a \times 1/a = 1$.

The probability of any particular temperature in the range decreases linearly as we move away from 20°C until it reaches zero at the ±2°C limits.

The standard deviation for the triangular distribution, i.e. the standard uncertainty, is $a/\sqrt{6}$, in this case $2/2.45 = 0.82$ °C.

9.4.1 Rectangular v/s triangular distribution

Often the choice to be made, in evaluating an uncertainty, is as to whether the rectangular or triangular distribution is most appropriate. In case of the effect of temperature variation on the uncertainty of the volume dispensed by 5 cc pipette discussed above a rectangular distribution was assumed, i.e. that all temperatures between 15°C and 25°C were equally likely.

The volume variation will also have a rectangular distribution. The half width of this distribution, derived from the calibration tables, is 0.005 cc for a 5 cc pipette. Hence the standard uncertainty is $0.005/\sqrt{3} = \pm 0.003$ cc.

If the laboratory were air conditioned with a target of 20°C and reasonable time were allowed to enable any solutions which were to be pipetted to equilibrate to the lab temperature. A view might be taken that triangular distribution is more appropriate since the probability of the temperature near 20 °C would be higher than the probability of

temperature at the 15 °c and 25 °c extremes. This would then make the standard uncertainty in the volume due to temperature effects $0.005/\sqrt{6} = 0.002$ cc.

In practice the difference between the standard uncertainty compounded from rectangular and triangular distributions with the same width is small; the difference between dividing by $\sqrt{3} = 1.73$ and $\sqrt{6} = 2.45$. This means that, unless the uncertainty being dealt with is a major contributor to the combined uncertainty it may not be worth spending time to arrive at defensible conclusion to use triangular distribution.

The rectangular distribution can always be justified as it represents the worst case scenario.

9.4.2 Conversion to Standard deviation

Uncertainty information from rectangular distribution , $U = a/\sqrt{3}$

Uncertainty information from triangular distribution , $U = a/\sqrt{6}$ For uncertainty associated from single result , use relative standard deviation RSD. $U = K \times RSD \times C$ (where C is a single result)

10. Case study -2

10.1 Uncertainties Associated in pipette and combining them

i. We have seen above that for the tolerance of a 5 cc class B pipette is ± 0.03 cc, the standard uncertainty in the 5.00 cc capacity pipette is $0.03/\sqrt{3} = 0.03/1.732 = 0.017$ cc.

ii. Calibration refers to 20 degree centigrade but when pipette is not used under the same temp., it will contribute additional uncertainties due to:

Coefficient of thermal expansion for pipette

Coefficient of thermal expansion for liquid being pipetted

Temperature range in lab

For class B pipette, the temperature coefficient for a borosilicate pipette and water over a temperature range of 15 to 25 °c the uncertainty in the measurement is ± 0.003 cc.

iii. Random uncertainties arise as even the same operator will dispense slightly different volumes each time pipette is used. Meniscus, number of taps on emptying may vary. In addition, each operator will have different technique. To arrive at it involves ten different operators, pipetting ten times water and weighing on four place- balance. Calculate mean and standard deviation. This would give a measure of uncertainty in the volume measurement in the laboratory due to random factors. Assuming that uncertainty due to weighing and temperature are negligible. This exercise will result in standard deviation of 0.01 cc, for pipetting 5 c.c. In this case , we have the following contributions to uncertainty:

The calibration Uncertainty in the pipette, ± 0.017 cc

The Uncertainty due to temperature variation ± 0.003 cc

The Uncertainty due to random effect ± 0.01 cc

10.2 Combining uncertainties

Example-4: Suppose in the above example, the quoted uncertainty is at 90% level of confidence. The standard uncertainty is then: $U(m) = 300 / 1.64 = 182.9 \text{ mg}$ (use table -1 to get 1.64 at 90% level of confidence)

Example-5: A calibration certificate states that the resistance of a standard resistor, R_s of a nominal value 10Ω is $10.000742 \Omega \pm 129 \mu\Omega$ at 23°C and that the quoted uncertainty of $129 \mu\Omega$ defines an interval having a level of confidence of 99%. Table 1 indicates a coverage factor of 2.58 at 99% confidence level., and equation-4

U (standard uncertainty) = $\sqrt{\sum (x_i - \bar{x})^2 / (n-1) \cdot K}$

$\sqrt{\sum (x_i - \bar{x})^2 / (n-1)}$ = standard deviation = $U(R_s) = 129 / 2.58 = 50 \mu\Omega$

Relative standard deviation = Standard deviation/nominal value

Relative standard deviation = Standard deviation/nominal value

Example-6 : A calibration certificate states that the length of a standard slip gauge (SG) of nominal value 50 mm is 50.000002 mm. The uncertainty of this value is 72 nm, at a confidence level of 99.7%

U (standard uncertainty) = $C \times$ Standard uncertainty

Standard uncertainty of slip gauge is = $72 \text{ nm} / 3 = 24 \text{ nm}$ (3 is the coverage factor K , at 99.77 confidence level)

Example-7 : The manufacturer's specification for a 100 ml class A volumetric flask is $\pm 0.08 \text{ ml}$. What is the standard uncertainty.

Answer: 0.046 ml (rectangular distribution, $0.08 / \sqrt{3}$)

Example-8: The manufacturer's specification for a 2 ml pipette is $\pm 0.01 \text{ ml}$. What is the standard uncertainty

Answer: 0.0058 ml (rectangular distribution, $0.01 / \sqrt{3}$)

Example-9: The calibration certificate for a four figure balance states that the measurement uncertainty is $\pm 0.0004 \text{ g}$ with a level confidence of 95%. What is the standard uncertainty

Answer: 0.0002 g (95% confidence interval- divided by 1.96)

Example 10: The purity of a compound as given in the label of bottle is 99.9 ± 0.1 . Determine the standard uncertainty in the purity of the compound.

Answer: 0.058% (rectangular distribution, $0.1 / \sqrt{3}$)

Example-11: A calibration weight is certified as $10.00000 \text{ g} \pm 0.04 \text{ mg}$ with a level confidence of at least 95%. What is the standard uncertainty in the weight?

Answer: 0.02 mg (95% confidence interval- divided by 1.96)

Example-12 : The standard deviation of repeat weighing of a 0.3g check weight is 0.00021g. What is the standard uncertainty of a single weighing?

Answer: 0.00021g (already a standard deviation, no conversion necessary)

Example-13: A calibration certificate for 25 ml class A pipette quotes an uncertainty of 0.03 ml. The reported uncertainty is based on a standard uncertainty multiplied by a coverage factor $k=2$, providing a confidence level of 95%. What is the standard uncertainty in the volume of the liquid delivered by the pipette.

Answer: 0.015 ml (divided by stated coverage factor i.e.2)

13. Possible sources of Uncertainty in a chemical lab

Uncertainty in the volume of liquid in a flask

Uncertainty in the volume of liquid delivered by a pipette

Uncertainty in weighing

Uncertainty in concentration of the solution

Example-14 Uncertainty in the volume of liquid in a flask

Contribution to the Uncertainty in the volume of liquid in a flask is from:

The Uncertainty in the volume of liquid in a flask is due to the very fact that each time you fill up to the mark of the flask the measured quantity of the liquid will differ. To evaluate this standard deviation ten fills of organic solvent are weighed using the 100 CC class A volumetric flask. The standard deviation is calculated by following the method as described in example 1, and for this flask it is (say) $U_p = 0.01732$ ml.

The manufacturer's specification for flask ± 0.08 ml Assuming it to be rectangular distribution $U_c = 0.08/\sqrt{3} = 0.046$ ml

The possible temperature difference between the laboratory temperature and the flask calibration temperature is estimated as $\pm 3^\circ$ with 95% confidence. The Coefficient of volumetric expansion of organic solvent is $1 \times 10^{-3}/^\circ\text{C}$. Uncertainty due to possible temperature difference between

laboratory temperature and the flask temperature is estimated as

Combining the three standard uncertainties = $\sum U^2_v + U^2_c + U^2_t$

$U_v = \sqrt{0.01732^2 + 0.046^2 + 0.153^2} = 0.16$ ml

Example-15. Uncertainty in the volume of liquid delivered by a pipette

a. Contribution to the Uncertainty in the volume of liquid in a flask is from.

The Uncertainty in the volume of liquid delivered by the pipette is due to the very fact that each time you fill up to the mark of the pipette the measured quantity of the liquid will differ. To evaluate this standard deviation ten fills of liquid are weighed using the 2CC class A pipette.

The standard deviation is calculated by following the method as described in example 14, is (say) $U_v = 0.0016$ ml.

b. Contribution due stated tolerance for the pipette is ± 0.01 ml.

The coefficient of volume expansion for organic solvent is 1×10^{-3} per $^{\circ}$ c. The uncertainty in calibration of pipette, is calculated from manufacturer's specification which is treated as rectangular distribution $U_c = 0.01 / \sqrt{3} = 0.0058$ ml

c. The manufacturers due to possible difference between the laboratory temperature and the pipette calibration temperature is estimated as $\pm 3^{\circ}$ with 95% confidence. The Coefficient of volumetric expansion of organic solvent is $1 \times 10^{-3} / ^{\circ}$ c. Uncertainty due to possible difference between laboratory temperature and the pipette calibration temperature is estimated as

$$U_t = 1 \times 1 \times 10^{-3} \times 3 / 1.96 = 0.00153 \text{ ml.}$$

$$U_v = \sqrt{0.0016^2 + 0.0058^2 + 0.00153^2} = 0.0067 \text{ ml}$$

Example -16 Uncertainty in weighing-

Contributors to the Uncertainty in weighing

a. The balance calibration certificate states a measurement uncertainty of ± 0.0004 gm with a level of confidence of not less than 95%. This converted to standard uncertainty by dividing by 1.96, $U_c = 0.0004 / 1.96 = 0.0002 \text{ g} = 0.2 \text{ mg}$

b. Replicate weightings of a 100 mg check weigh on the 4 figure balance has a standard deviation of $0.000041 \text{ g} = 0.041 \text{ mg}$

Combining uncertainties $U_w = \sqrt{0.2^2 + 0.041^2} = 0.201 \text{ mg}$

Example 17. Uncertainty in concentration of the solution

An internal standard solution is prepared by dissolving approximately 100.0 mg of material (weighed on a 4-figure balance) in an organic solvent and making it 100 ml in a volumetric flask.

Q-1 Calculate the concentration of solution in mg/L

Q-2 What is the standard uncertainty of the solution Concentration.

Q-3 What is the expanded uncertainty? Where $k=2$

Given data: 100.5 mg of material was weighed out, in balance & its calibration certificate states a measurement uncertainty of $\pm 0.0004 \text{ g}$, at 95% confidence level.

i. The standard uncertainty associated with weight was calculated above in Exercise-3 $U_w = 0.210 \text{ mg}$

ii. The purity of the material quoted by the supplier as $99.9 \pm 0.1\%$ standard deviation = $0.1 / \sqrt{3} = 0.058\%$ (rectangular distribution,)

iii. The standard uncertainty for the volume in 100 ml flask was calculated in exercise-1 and is = 0.16 ml.

Concentration of Solution = weight/ volume, $C = W/V$

The concentration of the solution = weight of material x purity/volume $100.5 \times 99.9/100$, this value is per 100 cc,

& to get per 1000 cc multiply by 10, the answer is = 10004.0 ml/L

Answer-1: 1004.0 mg/L

Standard uncertainty of the solution Concentration

(By adding individual uncertainties on account of balance, purity &, volume from calculations made & using the rule to combine)

Standard uncertainty of the solution concentration is given by

$$u(c) / C = \sqrt{(u(w)/w)^2 + (u(v)/v)^2 + (u(p)/p)^2} = 1004 \times \sqrt{(0.208/100.5)^2 + (.00058/0.999)^2 + (0.16/100)^2} = \pm 2.69 \text{ mg/L}$$

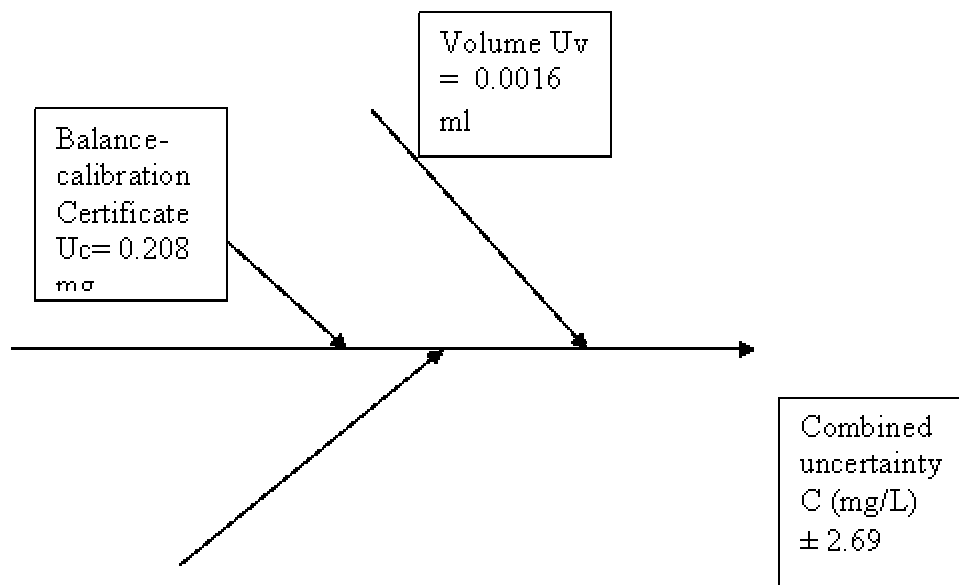
Answer -2. Standard uncertainty of the solution is = ± 2.69 mg/L

The expanded uncertainty of the solution Concentration :

U (expanded uncertainty) = 'K.' X. 'SD' (taking K=2) ,

Answer-3: Expanded uncertainty = $U_c = 2 \times 2.69 = \pm 5.38$ mg/l

Concentration is therefore quoted as 1004 ± 5.4 mg /L , where k=2 and the level of confidence is about 95%.



14. Cause and effect analysis (also called fish diagram)

$$u(c)/C = \sqrt{(u(w)/w)^2 + (u(v)/v)^2 + (u(p)/p)^2}$$

15. References

ISO-(GUM) "Guide to the expression of uncertainty in measurement".
NABL Guidelines for estimation and expression of un-certainty in measurement
Two-day training- Dr Bernard King
Evaluating uncertainties for laboratories Dr Alan G Rowley
EURACHEM / CITEC GUIDE Quantifying uncertainty in Analytical Measurements

. What is uncertainty?

I used to be uncertain - now I'm not so sure. In ordinary use the word 'uncertainty' does not inspire confidence. However, when used in a technical sense as in 'measurement uncertainty' or 'uncertainty of a test result' it carries a specific meaning. It is a parameter, associated with the result of a measurement (eg a calibration or test) that defines the range of the values that could reasonably be attributed to the measured quantity. When uncertainty is evaluated and reported in a specified way it indicates the level of confidence that the value actually lies within the range defined by the uncertainty interval.

2. How does it arise?

Any measurement is subject to imperfections; some of these are due to random effects, such as short-term fluctuations in temperature, humidity and air-pressure or variability in the performance of the measurer. Repeated measurements will show variation because of these random effects. Other imperfections are due to the practical limits to which correction can be made for systematic effects, such as offset of a measuring instrument, drift in its characteristics between calibrations, personal bias in reading an analogue scale or the uncertainty of the value of a reference standard.

3. Why is it important?

The uncertainty is a quantitative indication of the quality of the result. It gives an answer to the question, how well does the result represent the value of the quantity being measured? It allows users of the result to assess its reliability, for example for the purposes of comparison of results from different sources or with reference values. Confidence in the comparability of results can help to reduce barriers to trade. Often, a

result is compared with a limiting value defined in a specification or regulation. In this case, knowledge of the uncertainty shows whether the result is well within the acceptable limits or only just makes it.

Occasionally a result is so close to the limit that the risk associated with the possibility that the property that was measured may not fall within the limit, once the uncertainty has been allowed for, must be considered. Suppose that a customer has the same test done in more than one laboratory, perhaps on the same sample, more likely on what they may regard as an identical sample of the same product. Would we expect the laboratories to get identical results? Only within limits, we may answer, but when the results are close to the specification limit it may be that one laboratory indicates failure whereas another indicates a pass. From time to time accreditation bodies have to investigate complaints concerning such differences. This can involve much time and effort for all parties, which in many cases could have been avoided if the uncertainty of the result had been known by the customer.

4. What is done about it?

The standard ISO/IEC 17025:2005 [General requirements for the competence of testing and calibration laboratories] specifies requirements for reporting and evaluating uncertainty of measurement. The problems presented by these requirements vary in nature and severity depending on the technical field and whether the measurement is a calibration or test.

Calibration is characterized by the facts that:

1. repeated measurements can be made.
2. uncertainty of reference instruments is provided at each stage down the calibration chain, starting with the national standard and
3. customers are aware of the need for a statement of uncertainty in order to ensure that the instrument meets their requirements.

Consequently, calibration laboratories are used to evaluating and reporting uncertainty. In accredited laboratories the uncertainty evaluation is subject to assessment by the accreditation body and is quoted on calibration certificates issued by the laboratory.

The situation in testing is not as well-developed and particular difficulties are encountered. For example, in destructive tests the opportunity to repeat the test is limited to another sample, often at significant extra cost and with the additional uncertainty due

to sample variation. Even when repeat tests are technically feasible such an approach may be uneconomic. In some cases a test may not be defined well enough by the standard, leading to potentially inconsistent application and thus another source of uncertainty. In many tests there will be uncertainty components that need to be evaluated on the basis of previous data and experience, in addition to those evaluated from calibration certificates and manufacturers, specifications. International and accreditation aspects.

Accreditation bodies are responsible for ensuring that accredited laboratories meet the requirements of ISO/IEC 17025. The standard requires appropriate methods of analysis to be used for estimating uncertainty of measurement. These methods are considered to be those based on the Guide to the expression of uncertainty of measurement, published by ISO and endorsed by the major international professional bodies. It is a weighty document and the international accreditation community has taken up its principles and, along with other bodies such as EURACHEM/CITAC, has produced simplified or more specific guidance based on them. Accreditation bodies are harmonizing their implementation of the requirements for expressing uncertainty of measurement through organizations such as the European co-operation for Accreditation (EA) and the International Laboratory Accreditation Co-operation (ILAC).

5. How is uncertainty evaluated?

Uncertainty is a consequence of the unknown sign of random effects and limits to corrections for systematic effects and is therefore expressed as a quantity, i.e. an interval about the result. It is evaluated by combining a number of uncertainty components. The components are quantified either by evaluation of the results of several repeated measurements or by estimation based on data from records, previous measurements, knowledge of the equipment and experience of the measurement.

In most cases, repeated measurement results are distributed about the average in the familiar bell-shaped curve or normal distribution, in which there is a greater probability that the value lies closer to the mean than to the extremes. The evaluation from repeated measurements is done by applying a relatively simple mathematical formula. This is derived from statistical theory and the parameter that is determined is the standard deviation.

Uncertainty components quantified by means other than repeated measurements are also expressed as standard deviations, although they may not always be characterized by the normal distribution. For example, it may be possible only to estimate that the value of a quantity lies within bounds (upper and lower limits) such that there is an equal probability of it lying anywhere within those bounds. This is known as a rectangular

distribution. There are simple mathematical expressions to evaluate the standard deviation for this and a number of other distributions encountered in measurement. An interesting one that is sometimes encountered, eg in EMC measurements, is the U-shaped distribution.

The method of combining the uncertainty components is aimed at producing a realistic rather than pessimistic combined uncertainty. This usually means working out the square root of the sum of the squares of the separate components (the root sum square method). The combined standard uncertainty may be reported as it stands (the one standard deviation level), or, usually, an expanded uncertainty is reported. This is the combined standard uncertainty multiplied by what is known as a coverage factor. The greater this factor the larger the uncertainty interval and, correspondingly, the higher the level of confidence that the value lies within that interval. For a level of confidence of approximately 95% a coverage factor of 2 is used. When reporting uncertainty it is important to indicate the coverage factor or state the level of confidence, or both.

6. What is best practice?

Sector-specific guidance is still needed in several fields in order to enable laboratories to evaluate uncertainty consistently. Laboratories are being encouraged to evaluate uncertainty, even when reporting is not required; they will then be able to assess the quality of their own results and will be aware whether the result is close to any specified limit. The process of evaluation highlights those aspects of a test or calibration that produce the greatest uncertainty components, thus indicating where improvements could be beneficial. Conversely, it can be seen whether larger uncertainty contributions could be accepted from some sources without significantly increasing the overall interval. This could give the opportunity to use cheaper, less sensitive equipment or provide justification for extending calibration intervals.

Uncertainty evaluation is best done by personnel who are thoroughly familiar with the test or calibration and understand the limitations of the measuring equipment and the influences of external factors, eg environment. Records should be kept showing the assumptions that were made, eg concerning the distribution functions referred to above, and the sources of information for the estimation of component uncertainty values, eg calibration certificates, previous data, experience of the behavior of relevant materials. Statements of compliance - effect of uncertainty

This is a difficult area and what is to be reported must be considered in the context of the client's needs. In particular, consideration must be given to the possible consequences and risks associated with a result that is close to the specification limit. The uncertainty may be such as to raise real doubt about the reliability of pass/fail statements. When uncertainty is not taken into account, then the larger the uncertainty, the greater are the chances of passing failures and failing passes. A lower uncertainty is usually attained by using better equipment, better control of environment, and ensuring consistent performance of the test.

For some products it may be appropriate for the user to make a judgment of compliance, based on whether the result is within the specified limits with no allowance made for uncertainty. This is often referred to as shared risk, since the end user takes some of the risk of the product not meeting specification. The implications of that risk may vary considerably. Shared risk may be acceptable in non-safety critical performance, for example the EMC characteristics of a domestic radio or TV. However, when testing a heart pacemaker or components for aerospace purposes, the user may require that the risk of the product not complying has to be negligible and would need uncertainty to be taken into account. An important aspect of shared risk is that the parties concerned agree on the uncertainty that is acceptable; otherwise disputes could arise later.

Conclusion:

Uncertainty is an unavoidable part of any measurement and it starts to matter when results are close to a specified limit. A proper evaluation of uncertainty is good professional practice and can provide laboratories and customers with valuable information about the quality and reliability of the result. Although common practice in calibration, there is some way to go with expression of uncertainty in testing, but there is growing activity in the area and, in time, uncertainty statements will be the norm.

Links:

http://www.ilac.org/documents/pub_ilac-g17.pdf

<http://www.measurementuncertainty.org/mu/QUAM2000-1.pdf>