The development of a pharmaceutical product requires a broad spectrum of scientific expertise to lead it through the complex pathway from discovery through characterization of quality, efficacy and safety, which are the hallmarks of a successful drug product. A company must be highly proactive in setting targets for appraising and selecting a compound that has the highest probability of success. In addition, the compound and its therapeutic use must be consistent with the research and marketing goals of the company in order to leverage existing resources and experience. To ensure scientific and commercial success, it is critical to understand the drug development process (Fig.1.1) and the myriad tasks and milestones that are vital to a comprehensive development plan.

The ability to assure the physical and chemical properties of an active pharmaceutical ingredient, drug product or nutraceutical is critical for regulatory approval and therapeutic success. The effective chemistry, manufacturing and controls process is necessary for an efficient and comprehensive development strategy. The major challenges for the manufacturing and control component of drug development is to assure the chemical and physical properties of the compound and product are monitored at all critical phases of the pathway. This process matches the scientific and analytical tasks to the manufacturing and commercialization strategy (Table1.1).

In recent years, the International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) has adopted scientific standards for quality control monitoring [1]. These standards are the basis of most regulatory guidelines, including those published by the Food and Drug Administration [2]. Key steps on the path include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and
purity of ingredients, as well as those formulated products. Stability testing facilitates the establishment of recommended storage conditions, determination of retest periods and definition of acceptable shelf life. These data play a key role in determining labeling requirements, as well as in the development and monitoring process.

Stability testing is performed on drug substances and products at various stages of product development (Table 1.2). In early stages, accelerated stability testing (at relatively high temperatures and/or humidities) is used as a “worst case” evaluation to determine what types of degradation products may be found after long-term storage. In preformulation studies, interactions between excipients and the drug substance are studied under stress conditions to access compatibility.

The design of a complete stability-testing programme for a drug or nutraceutical product is based upon an understanding of the behaviour, properties and stability of the drug substance or active ingredient and the experience gained from preformulation studies and early clinical formulations. Products are analysed at specific intervals to evaluate a variety of parameters, such as the identity of the active ingredient, potency, measurement of degradation products, dissolution time, physical properties and appearance. Samples from production lots of approved products are retained for stability testing and for comparison testing in the case of product failure. Testing of retained samples alongside returned samples is key to ascertaining whether the product failure was manufacturing or storage related.

The objective of analytical testing during preclinical evaluation and Phase I clinical development is to evaluate the stability of the investigational formulations used in initial clinical trials, to obtain information needed to develop a final formulation and to select the most appropriate container and closure (e.g.,
compatibility studies of potential interactive effects between a drug substance and other components). Information from the experiments listed in Table 1.2 under discovery to Phase I is summarized in the investigational new drug application (IND) with the initiation of Phase I clinical trials. When the delivery mechanism of the drug is an integral part of creating the therapeutic effect and must be used in the Phase I trials, formulation data, container closure data and corresponding short-term accelerated stability data should be included in the IND prior to Phase I trials.

Analysis studies on formulations should be underway by the end of Phase II and the stability protocol for study of both the drug substance and drug product should be defined. This will help assure that analytical chemistry data generated during Phase III are appropriate for submission. Prior to Phase I, stability of the drug substance and the formulation to be used must be evaluated. Impurities from the manufacturing and degradants that form are quantitated and tracked to ensure safety prior to moving into the Phase I clinical trials and continuity of material used for laboratory safety testing and clinical trials.

Stability testing done during Phase III studies focuses on testing final formulations in the proposed packaging produced at the manufacturing site. It is recommended that the stability protocol is defined prior to the initiation of Phase III studies. In this regard, consideration should be given to establish appropriate linkage between the non-clinical and clinical batches of the drug substance and drug product and those of the primary stability batches in support of the proposed expiration-dating period. Factors to be considered include the source, quality and purity of various components of the drug product, manufacturing process and production facility for the drug substance and the drug product, as well as use of same
containers. Data obtained on tests done under controlled conditions replicating conditions recommended for long-term storage and slightly elevated temperatures are used to determine a product’s shelf life and expiration dates.

The stability of a product may be defined as the extent to which a product retains, within specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging. Stability testing provides evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. These studies are designed to determine if a drug substance will remain within specifications during its shelf life if stored under recommended storage conditions.

Stability testing focuses on the chemical (i.e., integrity, potency, degradation) and physical properties (e.g., appearance, hardness, particle size, solubility) of active pharmaceutical ingredients (API) and products. In addition, microbiological testing is done to ensure the substance and product maintain their resistance to microbial and bacterial growth. Assuring the physical/chemical properties and effectiveness properties of a pharmaceutical is critical for labeling and marketing purposes. A wide range of testing is used to evaluate and verify the identity, potency and availability of the API in the product (Table 1.3). Stability testing is done at all phases of the development, production and marketing process for quality control and monitoring purposes. Early in the development of the drug product, purposeful degradation studies are done as a means to predict possible degradation pathways of an API. This information is used in the validation of stability indicating analytical methods and in pre-formulation studies. Degradation studies include stress conditions such as heat, oxidative, light, acidic conditions,
basic conditions and heat/humidity. Testing to assure that products meet specifications for the presence of degradation products and impurities are usually intensive chromatographic separations with detection down to the 0.01% levels. Typically, impurities and degradation products that are 0.1% and above need to be evaluated for identity and chemical structure. The level of the impurities allowed depends on the toxicity of the impurity and the daily dose levels of the drug. Identity information on the stability of a drug substance under defined storage conditions is an integral part of the systematic approach to stability evaluation. Stress testing helps to determine the intrinsic stability characteristics of a molecule by establishing degradation pathways to identify the likely degradation products and to validate the stability indicating power of the analytical procedures used.

The severe conditions encountered during distribution are covered by stress testing of definitive batches of the drug substance. Stress testing provides data on forced decomposition products and mechanisms. These studies establish the inherent stability characteristics of the molecule (e.g., degradation pathways) and lead to identification of degradation products and support the suitability of the proposed analytical procedures. The detailed nature of the studies will depend on the individual drug substance and type of drug product.

Testing is carried out on a single batch of a drug substance and includes the effects of temperatures in 10°C increments above the accelerated temperature test condition and humidity, where appropriate (e.g., 75% or greater). In addition, one must evaluate oxidation and photolysis on the drug substance, plus its susceptibility to hydrolysis across a wide range of pH values when in solution or suspension. Photostability (i.e., light) testing is an integral part of stress testing. Some
degradation pathways can be complex and, under forced conditions, decomposition products may be observed that are unlikely to be formed under accelerated or long-term testing. This information is useful in developing and validating suitable analytical methods, but may not be necessary to examine specifically for all degradation products if it has been demonstrated that in practice these are not formed. Information obtained from photostability is key in choosing appropriate container/closure system.

The route of administration and delivery system used are key components to the successful development of new drugs and therapies. In addition, these choices have a significant impact on the scientific and regulatory aspects of a stability protocol. The diversity of testing needed for all dosage forms and delivery systems requires a broad range of expertise and methodologies.

In general, all dosage forms are evaluated for appearance, assay and degradation products. Additional tests (i.e., potency) are needed for specific dosage forms. For example, sterility is needed for sterile products but not for tablets or capsules. In addition, not every test will be performed at each time point.

Purity, a metaphysical concept, has always been considered as an essential factor in ensuring drug quality. Absolute purity does not exist and the degree of purity of a product is only a reflection of the analytical techniques used for its assessment. All pharmaceutical substances unavoidably contain impurities resulting from many sources like raw materials and reagents, as reaction byproducts and through degradation during manufacture and storage or extraneous contamination. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. The different pharmacopoeias, such as the British Pharmacopoeias (BP) and the United States Pharmacopoeias
(USP) are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations. Also, the ICH has published guidelines on impurities in new drug substances [3], products [4], and residual solvents [5]. In addition, Ahuja [6] and Gorog [7] have published books covering different aspects of impurities, including the governmental regulations and guidelines and the identification and monitoring of impurities found in drug products.

A number of recent articles [8-10] have described a designed approach and guidance for identifying process related impurities and degradation products using mass spectrometry, NMR, high performance liquid chromatography, fourier transform ion cyclotron resonance mass spectrometry and tandem mass spectrometry for pharmaceutical substances. In general, according to ICH guidelines on impurities in new drug products [4], identification of impurities below the 0.1% level is not considered to be necessary unless the potential impurities are expected to be unusually potent or toxic. There are two types of impurities in medicines:

- impurities associated with active pharmaceutical ingredients
- impurities that are created during formulation and/or with aging or that are related to the formulated forms.

According to ICH guidelines [4], impurities associated with APIs are classified into three classes for regulatory purpose as organic, inorganic and residual solvents.

- Organic impurities may arise from starting materials (most often from isomeric impurities), synthetic intermediates (incomplete reaction or excess reagent used) and degradation product which may depend on alteration in reaction conditions such as temperature, pH or in storage condition (hydrolysis, ring opening etc.).
Inorganic impurities present in pharmaceutical products originate from the equipment used and from reagents, catalysts, drying agents and filter aids.

Residual solvents and other volatile impurities must be detected and assayed, not only because of their potential toxicity and deleterious environmental effects, but also because they can impart undesirable organoleptic characteristics to drugs.

The increasing emphasis on the pharmacokinetics, bioavailability and therapeutic monitoring of drug has placed a heavy burden on drug analyst. Thanks to advances in analytical instrumentation and a greater understanding of metabolism, we can now detect drugs at concentrations that were once undetectable. Because of the vast number of drugs introduced every year, more and more methods for drugs and metabolite determination are being developed either for routine or research use. Analytical procedures are used throughout the drug development and manufacturing of drug substances and drug products.

The application of increasingly sophisticated methods for structure analysis by spectroscopy, isotopic labeling, automated quantitative analysis and separation by chromatography and other partition procedures opened avenues of study of minute amounts of biochemicals. Instrumental analysis opened many doors to scientific progress in support of both traditional core areas of chemistry and new ones like biotechnology, material chemistry, environmental chemistry, chemical toxicology and small domain chemistry.

In the field of drug analysis, volumetric methods, either direct or indirect, have their own importance due to their inherent simplicity. Owing to this, a large number of official methods for the determination of pharmaceuticals in the pharmacopoeias are based on titrimetry. Though it is the oldest technique in the
market now but still have their recognition in the field of scientific research. Recently titrimetric methods have been used for the determination of albendazole [11], gatifloxacin [12], promethazine theodate [13] and nizatidine [14] in commercial dosage forms.

Chromatographic methods have many applications in trace analysis. The different types of chromatography such as thin layer chromatography, column chromatography, high performance liquid chromatography, gas chromatography and capillary electrochromatography have most frequent applications in the field of pharmaceutical as well as biomedical analyses.

Thin layer chromatography (TLC) is simple, rapid, inexpensive and widely used for the separation and identification of pharmaceuticals. A TLC method has been developed for the determination of oxysterols in plasma using plates coated with either RP-C18 or silica gel F_34 [15]. This technique has also been used to analyse celecoxib in commercial dosage forms [16]. The contents of gestodene and cyproterone have been determined in raw material and dosage forms by thin layer chromatography [17].

High performance thin layer chromatography (HPTLC) is a fast separation technique and flexible enough to analyse different kinds of samples. Review papers [18,19] by Renger have discussed the potential of TLC and HPTLC in pharmaceutical analysis. The advantages associated with this technique are as follows:

- simple to handle the instruments.
- short analysis time to analyse complex or crude sample with minimum sample clean up.
• evaluate the entire chromatogram with a wide variety of techniques and parameters without time constraints.
• simultaneous but independent development of multiple sample and standards on each plate, leading to an increased reliability of results.
• robustness for easy transfer of samples.

Several drugs have been successfully investigated by HPTLC in pharmaceutical preparations [20-23].

High performance liquid chromatography has gained lots of attention recently in the field of drug analysis in both dosage forms and biological fluids because of its simplicity, high specificity and sensitivity. For example, captopril can be measured in pharmaceutical tablets by anion exchange HPLC using indirect photometric detection [24]. The method is also used in the determination of alprazolam [25] and buspirone [26] in pharmaceutical formulations. Dexamethasone has been estimated by HPLC using isocratic reversed phased procedure in cream formulations [27]. This method can also be used to measure impurities [28,29], degradation products [30,31] and product preservatives at levels between 0.05 and 0.1%. HPLC assay procedures have been developed for cephalexin and cefadroxil, cefaclor and cefataxim in pharmaceuticals [32] and for cefixime trihydrate in bulk drugs [33].

Because of the specificity and sensitivity afforded by gas chromatography, it has been widely used for the detection and determination of trace level contaminants in pure substances and also in pharmacokinetic studies. The use of mass spectrometer as the gas chromatographic detector provides additional sensitivity and specificity over other analytical methods [34]. A number of papers have been published on the application of gas chromatography in pharamaceutical analysis
both in dosage forms and biological fluids [35-37]. The method was also used for the determination of isotretinoin and its degradation products in pharmaceuticals [38].

In flow injection analysis (FIA) a quantity of dissolved sample, accurately measured, is injected or introduced into the carrier stream flowing through the system tubing, with or without additional changes like chemical reactions etc. occurring between the sample and the carrier. As the analyte passes continuously through the detector, a transient signal is generated and recorded. The main reasons for the success are the following advantages of FIA over conventional manual techniques:

- great precision due to mechanical performance of the assays.
- reduced labour costs due to automation.
- higher sample throughput due to mechanical performance of the assay.
- small sample and reagent consumption and waste generation due to smaller scale of the assay

This technique has been utilized successfully in the determination of some compounds of pharmaceutical interest [39-41]. Later on, FIA evolved in sequential injection analysis (SIA) [42]. The introduction of SIA brought computer control into flow injection, thus increasing the degree of automation and making the technique more versatile.

Capillary electrophoresis (CE) is an automated analytical technique that separates species by applying voltage across buffer filled capillaries. It is generally used for separating ions, which move at different speeds when the voltage is applied depending on their size and charge. The solutes are seen as peaks as they pass through the detector and the area of each peak is proportional to their concentration,
which allows quantitative determinations. Analysis includes purity determination assays, and trace level determinations. Pharmaceutical companies make extensive use of CE [43] and are widely accepted by regulatory authorities such as US food and drug administration [44-47]. CE methods have often replaced existing titration and ion-exchange chromatographic (IEC) methods because they are often faster, more efficient and cost effective. Another benefit is that the analysis performed on standard CE instrumentation and does not require extra IEC equipment, columns and reagents. One area where CE has distinct advantage over other separation techniques is chiral analysis. Chiral separations are among the most widely used application of CE [48]. Several reports have appeared on the application of this technique in different modes such as capillary zone electrophoresis [49], micellar electrokinetic chromatography [50], isotachophoresis [51], capillary electrokinetics [52], capillary gel electrophoresis [53], iso-electric focusing [54] and affinity capillary electrophoresis [55].

Electrochemical methods are concerned with the measurements of electrical quantities such as (i) voltage (V), (ii) current (I), (iii) charge (Q) and (iv) resistance (R) to gain information about the composition of the solution and the reaction kinetics of its components. Important electrochemical techniques such as amperometry, conductometry, potentiometry, coulometry, anodic and cathodic stripping voltammetry [56-58], differential pulse voltammetry and polarography have been used in drug analysis [59, 60]. Potentiometric titrations are also reported in the pharmacopoeias as the standard method for the determination of certain drug substances [61-63].

Spectroscopic methods are widely used for the assay of drugs in pharmaceutical formulations. The most commonly used technique for quantitative
analysis are ultraviolet (UV) and visible spectrophotometry. The simplicity of UV-spectrophotometry is best known and most widely accepted for qualitative and quantitative analyses. Conventional UV-spectrophotometric methods are simple, fast and economical and do not need any elaborate preparatory step prior to assay. However, these methods are inadequate when two or more drugs showing similar UV spectra in the presence of other components in the drug sample like excipients or decomposition products.

Photometric methods of analysis are performed in the visible region of light. These methods are usually based on the following aspects:

- oxidation-reduction process.
- complex-formation reaction.
- a catalytic effect.

In each type of reaction the absorbance of coloured compounds is measured. Usually, the analytes under investigation are colourless, they are reacted with suitable chemical reagents in order to convert them in coloured compounds. Simple colorimetric and UV methods continue to be popular for carrying out single component assays on a variety of formulated products. Representative examples of UV-visible methods of drug analysis for some pharmaceuticals that have been published [64-107] are given in Table 1.4.

In the course of this discussion, the importance of computer-aided spectrophotometric determination of multicomponent systems cannot be ignored. Pharmaceutical preparations are usually mixtures of the active principal and various excipients that absorb in the same region as the component of interest, thereby resulting in band overlap and impeding the use of the technique with simple calibration methods. The inception of microcomputers and spectrophotometers that
allow absorbance spectra to be expeditiously recorded at many wavelengths has enable the development of analytical methods based on the mathematical resolution of multivariate signals for the rapid quantitation of mixtures of analytes in control analyses. The application of multivariate calibration methods to spectral data in biomedical and pharmaceutical fields has acquired a routine nature [108-112]. Partial least square (PLS) has several advantages of using full spectra, which is critical for the spectroscopic resolution of complex mixture of analyte. It allows for a rapid determination of components usually with no need of a prior separation. An additional advantage of PLS is that calibration can be performed by ignoring the concentrations of all other components except the analyte of interest. The complementary use of PLS multivariate calibration and artificial neural network for the simultaneous spectrophotometric determination of three active components such as chlorpheniramine, nephazoline and dexamethasone in a pharmaceutical formulation has been successfully explored [113].

Derivative spectrophotometry is an analytical technique of great utility for both qualitative and quantitative information from spectra composed of unresolved bands. Although it was introduced for more than thirty years ago [114-118] and has advantages for the solution of specific analytical problems. The derivative methods have found applications not only in the UV-visible spectrophotometry [119], but also in infra-red [120], atomic absorption [121], flame emission spectrometry [122] and flourimetry [123]. The use of derivative spectrophotometry is not restricted to special cases, but may be of advantage whenever quantitative study of normal spectra is difficult. Its disadvantage is that the differentiation degrades the signal-to-noise ratio, so that some form of smoothing is required in conjunction with the differentiation [124,125].
Near infra red (NIR) spectroscopy is a technique which has found its way into pharmaceutical control laboratories in recent years for raw material identification, water analysis and other pharmaceutical analyses [126-129]. NIR has the potential to dramatically improve the quality of drug manufacturing. There are several advantages associated with this technique such as reduction in the cost of testing, require no reagents, associated reagents preparation steps, sampling preparation steps and generally require only one working analyst day to complete testing. The United State Pharmacopoeia has proposed guidelines [130,131] for this technique.

Nuclear magnetic resonance (NMR) spectroscopy has been mainly used for the elucidation and confirmation of structures. For the last decades, NMR methods have been introduced to quantitative analysis in order to determine the impurity profile of a drug, to characterize the composition of drug products in body fluids, in solid state measurements to provide the information and for micro-imaging to study the dissolution of tablets [132-136].

Fluorimetry and phosphorimetry find wide applications in quantitative studies of rates of degradation, metabolism and excretion of drugs where other analytical techniques are not sufficiently sensitive. A gradual increase in the number of papers on the potential application of fluorimetry and phosphorimetry in the field of pharmaceutical analysis has been noticed during the last few years [137-141].

In the recent period, analysts are much interested in coupling the chromatographic techniques with spectroscopic techniques. The advent of liquid-liquid chromatography with mass spectrometry [142-144], gas chromatography with tandem mass spectrometry and liquid chromatography-electrospray tandem mass spectrometry are good examples. Hirsch and coworkers have determined the
antibiotics using chromatography-electrospray tandem mass spectrometry [145]. Dielectric relaxation spectroscopy and X-ray powder diffractometry are also utilized for the identification of pharmaceuticals [146].

Kinetic automatic methods are good choices for drug analyses as they permit the sensitive, selective determination of many drugs within a few seconds with no pretreatment. Moreover, the instrumentation required is generally very simple. Kinetic methods rely on measurement of concentration changes (detected via signal changes) in a reactant (which may be the analyte itself) with time after the sample and the reagents have been mixed. The sample and reagent can be mixed manually or automatically and the kinetic curve (variation of analytical signal with time) can be recorded immediately. The slope of the straight initial portion of the kinetic curve gives the reaction rate, which is proportional to the analyte concentration (initial-rate method). The fixed-time method also frequently used to derive such a concentration, involves measuring the signal (the value of which depend on the analyte concentration) at a pre-set time. The fixed-time and initial-rate methods have been used more frequently for the determination of drugs in pharmaceutical formulations and biological fluids [147-149]. Kinetic is relevant to analytical chemistry in at least four respects:

- it allows the elucidation of the physical, chemical and physico-chemical mechanisms on which analytical processes are based and hence their rational optimisation.
- it facilitates the development of new analytical methods that are otherwise unattainable if the dynamic aspects are not dealt with.
- it is the foundation of reaction rate method.
it contributes to the improvement of significant analytical parameters such as sensitivity, selectivity and precision.

Whenever the question of mathematical and statistical treatment arises, the role of chemometrics cannot be ignored. Chemometrics is defined as “the chemical discipline that uses mathematical, statistical and other methods of formal logic to design or select optimal procedures and experiments and provide maximum chemical information by analysing chemical data” [150-152]. Chemometrics is the science that helps to make good use of information technology in the chemical analysis and to develop intelligent analysers i.e. that automatically select the correct method for a given problem, carry it out, validate it and interpret the results.
METHOD VALIDATION STRATEGY

Before an analytical method can be used for routine analysis, it must first be demonstrated that the method fulfills certain performance criteria. When this has been documented, the method is said to be validated. In order to address the performances of the analytical procedure adequately, the analyst is responsible to identify the relevant parameters, to design the experimental validation studies and define the appropriate acceptance criteria. The purpose of method validation is to establish that an accurate, precise and rugged method has been developed. The process for the development, validation and use of analytical method is shown in Fig.1.2.

The validation of analytical methods used in bioavailability, bioequivalence and pharmacokinetic studies in human and animals has been the subject of discussion in recent years [153-158]. Several International Organisations and Regulatory Authorities, which are involved in fixing the criteria for the validation, are listed in Table 1.5. It has been agreed that the following parameters must be considered for evaluation of method reliability and overall performances:

- confirmation of identity
- analyte stability
- selectivity/specificity
- precision and accuracy
- limit of detection and quantitation
- recovery
- robustness/ruggedness

The overall validation strategy consists of four components, which are prevalidation [159,160], proper validation [161,162], study proper and statistical analysis.
Confirmation of identity

In general analytical methods consist of a measurement stage, which may be preceded by an isolation stage. It is necessary to establish that the signal or reaction produced at measurement stage is only due to analyte and not due to something chemically or physically similar or arising as a coincidence. This is the confirmation of the identity, whether or not other compounds interfere with the measurement of the analyte will depend on the effectiveness of the isolation stage if it was part of the method, as well as the specificity/selectivity of the measurement stage.

Selectivity/Specificity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Selectivity should be ensured at the lower limit of quantification (LLOQ) [163-165]. The term ‘specific’ generally refers to a method that produces a response for single analyte.

Accuracy and Precision

The accuracy [166] of a method is defined as the closeness of agreement between the test results and the accepted reference value. This is sometimes termed trueness.

According to ICH, the precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions and may be considered at three levels:

- repeatability
- intermediate precision
- reproducibility
**Repeatability**

Repeatability is the precision obtained by independent test results with the same method on identical test material in the same laboratory by the same operator using the same equipment within short interval of time. It is also termed as intra-assay precision [167-168]. Repeatability is also sometimes termed as within run or within day precision.

**Intermediate Precision**

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment etc. [159]. The International Organization for Standardization (ISO) definitions used the term “M-factor different intermediate precision” where the M-factor expresses the number of factors (operator, equipment or time) that differ between successive determination [169]. Intermediate precision is sometimes also called between-run, between-day or inter-assay precision. Several approaches discussed in ICH guidelines are given in Table 1.6.

**Reproducibility**

Reproducibility is the precision obtained within the same method on identical test material in different laboratories with different operators using different equipments [170].

**Limit of detection and quantitation**

Limit of detection (LOD) determines the lowest amount of analyte that can be detected, as it (the analyte) yields instrumental response greater than a blank, but cannot be quantified. The common definition of LOD is the concentration (or quantity) of analyte that produces a signal, that exceeds the signal observed from a
blank by an amount equal to three times the standard deviation from the measurement on the blank [162].

A limit of quantitation (LOQ), defined as the analyte concentration for which the signal exceeds that for a realistic analytical blank by ten times the standard deviation, is often specified as the smallest analyte concentration that one should attempt to quantify [164]. Several approaches have been given in the ICH guidelines to determine the detection and quantitation limits (Table 1.7).

**Calibration range and Dynamic range**

The calibration range will be defined by the expected concentration in the samples and will be usually in the linear range of the detector for that analyte, where the determined response is directly proportional to the concentration interval over which acceptable accuracy, linearity and precision are obtained. In practice the range is determined using data from the linearity and accuracy studies.

Fig. 1.3 illustrates the definition of the dynamic range of an analytical method, which extends from the lowest concentration at which quantitative measurements can be made to the concentration at which the calibration curve departs from linearity (limit of linearity).

**Recovery**

The recovery is a measure of all efficiency of the method in detecting all the analyte of interest present in the original sample. Recovery should be as high as possible, but is more important that it (recovery) is constant within the calibration range [173].

**Robustness/Ruggedness**

ICH [174] defines the “robustness/ruggedness” of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variation in
method parameters and provides an indication of its reliability during normal usage [162-166]. Ruggedness is a measure for the susceptibility of a method to small changes that might occur during routine analysis like small changes of pH values, mobile phase composition, temperature etc. Full validation must not necessarily include ruggedness testing; it can, however, be very helpful during the method development/prevalidation phase, as problems that may occur during validation are often detected in advance. Ruggedness should be tested, if a method is supposed to be transferred to another laboratory [175].

STATISTICAL ANALYSIS

Recent trends [176-179] in the pharmaceutical analysis emphasize the use of statistical analysis and evaluation of the method against statistical parameters like correlation coefficient, variance, errors and confidence limits, limit of detection and sensitivity, standard deviation, ‘r’ and ‘F’ values, standard deviation of slope and intercept etc. A brief discussion is given below.

Correlation Coefficient

When using instrumental methods, it is often necessary to carry out a calibration process by using a series of samples (standard) each having a known concentration of analyte. Two statistical procedures should be applied to the calibration curve:

- test whether the graph is linear or in the form of a curve
- find the best straight line (or curve) through the data points

Linearity is often tested by correlation coefficient, ‘r’, which can be calculated for a calibration curve to ascertain the degree of correlation between the measured instrumental variable and the sample concentration.
\[
\begin{align*}
    r &= \frac{n \sum x_i y_i - \sum x_i \sum y_i}{\sqrt{n (\sum x_i^2 - (\sum x_i)^2)(\sum y_i^2 - (\sum y_i)^2)}} \\
\end{align*}
\]

where,

\(\bar{x}\) = mean of all the values of \(x_i\)

\(\bar{y}\) = mean of all the values of \(y_i\)

\(n\) = number of data points.

The maximum value of ‘r’ is 1. When this occurs there is exact correlation between the two variables (\(x\) and \(y\)). When the value of ‘r’ is zero (\(xy=0\)), there is complete independence of the variables. As a general rule, \(0.90 < r < 0.95\) indicates a fair curve, \(0.95 < r < 0.99\) as a good curve, and \(r > 0.99\) includes excellent linearity.

**Linear least squares**

The best straight line through a series of experimental points is that line for which the sum of the squares of the deviation of the points from the line is minimum. Besides determining a straight line, uncertainties in the use of calibration graph for analysis of unknown samples can be specified by the method of least squares. The equation of the straight line is

\[ A = a + bC \]

Where

\(A\)= instrumental response (i.e. absorbance), \(b\)=slope, \(a\)=intercept, and

\(C\)=concentration

To obtain the regression line \(A\) on \(C\), the slope ‘\(b\)’ of the line and the intercept ‘\(a\)’ on the \(y\)-axis are given by the following equations
\[ b = \frac{\sum x_i y_i - [\sum x_i \sum y_i]/n}{\sum x_i^2 - [\sum x_i^2]/n} \]

\[ a = \frac{\sum y_i - b \sum x_i}{n} \]

where

\( x_i \) = individual value of concentration

\( y_i \) = individual value of absorbance corresponding to \( x_i \)

\( n \) = number of data points in the calibration line

**Error in the concentration**

The error (\( S_c \)) in the determination of the concentration of the analyte in the given sample can be calculated using the relation:

\[ S_c = \frac{S_0}{b} \left[ 1 + \frac{1}{n} + \frac{(y - \bar{y})^2}{b^2 \sum (x - \bar{x})^2} \right]^{1/2} \]

where \( \bar{x} \) and \( \bar{y} \) are the average concentration and absorbance values, respectively, for ‘n’ standard solutions. Standard deviation of the calibration line (\( S_0 \)) is evaluated from the following equation:

\[ S_0 = \sqrt{\frac{\sum (y_i - \bar{y})^2}{n - 2}} \]

**Confidence limit for the slope and intercept**

It determines whether the slope and/or intercept of a line differ significantly from a particular or predicted value. It can be calculated as,

\[ b \pm t S_b \]  
(for slope)

and/or
\[ a \pm t \, S_a \]  
(for intercept)

where

\[ t = \text{tabulated} \ 't' \ \text{value at desired confidence level for (n-2) degrees of freedom.} \]

**Limits of detection and quantitation**

Limit of Detection (LOD)/ Limit of Quantitation (LOQ) are defined as the 3.3 and 10 fold, respectively of the standard deviation of the calibration line. The values are converted to a concentration by the slope of a corresponding calibration line.

\[
\text{LOD} = 3.3 \times \frac{S_0}{b} \\
\text{LOQ} = 10 \times \frac{S_0}{b}
\]

**Significance of testing**

An important property of an analytical method is that it should be free from the systematic error (bias). Determining bias involves analyzing one or more standard reference materials whose analyte concentration is known. However, random errors make it unlikely that, the measured amount will equal to the known amount even when no systematic errors are present. In order to decide whether the difference between the observed and standard values can be accounted for by random variation, a statistical test i.e. a significance test is used for the interpretation of analytical data.

- **Student's \(t\)-test:** Here comparison is made between two sets of replicate measurements made by two different methods; one is the test method while other is accepted (reference method).

\[
\pm t = \frac{\bar{x}_1 - \bar{x}_2}{S_p} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}
\]
where,

\[ x_1 = \text{mean from the test method} \]

\[ x_2 = \text{mean from the accepted (reference) method} \]

\[ n_1 \& n_2 = \text{number of measurements} \]

\[ S_p = \text{pooled standard deviation of the individual measurements of two sets} \]

is given by

\[
S_p = \sqrt{\frac{\sum (x_1 - \bar{x}_1)^2 + \sum (x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}}
\]

A statistical \( t \)-value is calculated and compared with a tabulated value for the given number of tests at the desired confidence level. If \( t_{cal} > t_{tab} \) then there is significant difference between the results obtained by the two methods at the given confidence level. But if \( t_{cal} < t_{tab} \) then there is no significant difference between the methods. It is an accuracy-indicating test.

- **F-Test:** This test indicates whether there is a significant difference between the two methods (i.e. the new method and the accepted reference method). It can be represented as,

\[
F = \frac{S_1^2}{S_2^2}
\]

where

\[ S_1 \text{ and } S_2 = \text{standard deviation of method 1&2.} \]

The calculated value of ‘\( F \)’ is compared with the tabulated standard value at the selected confidence level and degrees of freedom. If \( F_{cal} > F_{tab} \) then there is significant difference between the two methods. It is a precision indicating test.
Interval hypotheses

For practical purposes, the acceptable bias can be calculated statistically [180].

For example, a test method (method 2) is considered acceptable if its true mean value is within that of the reference method (method 1), i.e.

\[-0.02 \mu_1 < (\mu_2 - \mu_1) < 0.02 \mu_1\]

this can be written as

\[0.98 < \mu_2/\mu_1 < 1.02\]

which can be generalised to

\[\theta_L < \mu_2/\mu_1 < \theta_U\]

where \(\theta_L\) and \(\theta_U\) represents the lower and the upper acceptance limits, respectively when \(\mu_2\) is expressed as the proportion of the reference mean \(\mu_1\).

Statistically, \(\theta_L\) and \(\theta_U\) can be calculated from the relation

\[\theta^2 \left( \bar{x}_1^2 - \frac{S^2 \cdot t^2_{tab}}{n_1} \right) - 2\theta \bar{x}_1 \bar{x}_2 + \left( \bar{x}_2^2 - \frac{S^2 \cdot t^2_{tab}}{n_2} \right) = 0\]

The lower limit \((\theta_L)\) and the upper limit \((\theta_U)\) of confidence interval are obtained as

\[\theta_L = \frac{-b - \sqrt{b^2 - 4ac}}{2a}\]

\[\theta_U = \frac{-b + \sqrt{b^2 - 4ac}}{2a}\]

where,

\[a = \bar{x}_1^2 - \frac{S^2 \cdot t^2_{tab}}{n_1}\]

\[b = -2\bar{x}_1 \bar{x}_2\]
\[ c = \frac{\bar{x}_1^2 - \frac{S_p^2 t_{\text{tab}}^2}{n_2}}{n_1} \]

where \( \bar{x}_1 \) and \( \bar{x}_2 \) estimates of \( \mu_1 \) and \( \mu_2 \) based on \( n_1 \) and \( n_2 \) measurements respectively. \( S_p \) is the pooled standard deviation and \( t_{\text{tab}} \) is the tabulated one sided \( t \)-value, with \( n_1 + n_2 - 2 \) degrees of freedom at the specified level of significance.

**Testing for outliers**

When a series of replicate measurements of same quantity are made, one of the results will appear too different markedly from the other. There is then a great temptation to discard this “outliers” before calculating the mean and the standard deviation of the data or applying statistical tests to compare the data with other measurements. The best known method for this purpose is Dixon’s Q-test.

\[ Q = \frac{|\text{Suspected value - Nearest value}|}{|\text{Largest value - Smallest value}|} \]

If \( Q_{\text{cal}} \geq Q_{\text{tab}} \) at a given confidence level, then the outliers can be rejected.
CLASSIFICATION OF DRUGS

The drugs can be prepared synthetically or reconstituted from natural sources product. All the drugs having medicinal importance can be classified considering their structures and pharmacological properties.

**Chemical classification**

The drugs are classified according to their chemical structure and properties without taking the pharmacological action. In this class most of the drugs are having at least an organic substrate; further classification is done in the relevant manner.

**Pharmacological classification**

In this class the drugs are divided according to their action on the organism’s organ (viz. heart, brain, lymphatic system, respiratory system, endocrine system, central nervous system etc.) Hence these drugs are called cardiovascular, narcotics, analgesics, antibiotics, diuretics, and anaesthetics etc. Further classification of each group is done according to the therapeutic/pharmacological specificity with the relevant organ. A detailed classification of drugs based on pharmacological action on human organs has been given in Scheme 1.1.

The present thesis deals with the determination of captopril, lisinopril dihydrate, labetalol hydrochloride and perindopril erbumine in pharmaceutical formulations.
Captopril, 1-[(2S)-3-mercapto-2-methyl-propionyl]-L-proline, is a sulphahydrl containing angiotensin converting enzyme inhibitors (ACE) which reduces peripheral resistance and lowers blood pressure. It is widely used in the hypertensive ailments and in congestive heart failure treatment. Captopril lowers blood pressure, but in the short term magnitude of response is independent of Na\(^+\) status and the level of renin-angiotensin activity. A greater fall in blood pressure occurs in renovascular, accelerated and malignant hypertension. However, in the long term no correlation has been observed between plasma renin activity and intensity of fall in blood pressure due to captopril. Captopril induced hypotension is a result of decrease in total peripheral resistance. The arterioles dilate and compliance of larger arteries is increased. It is also used to treat kidney problems in some diabetic patients who use insulin to control their diabetes. Captopril was the first ACE inhibitor developed and was considered a break through both because of its novel mechanism of action and also because of the revolutionary development process.
Lisinopril dihydrate, 1-[n-[(s)-1-Carboxy-3-Phenylpropyl]-L-lysyl]-L-proline dihydrate, belongs to the group of medicines known as ACE inhibitors. It is a lysine derivative of analaprilat, which does not require hydrolysis to become active ACE inhibitor. Administration of lisinopril blocks the renin angiotensin-aldosterone axis and tends to reverse the potassium loss associated with the diuretics. The anti hypertensive effects of lisinopril have continued during long term therapy. Abrupt withdrawal of lisinopril has not been associated with a rapid increase in blood pressure; nor with a significant overshoot of pretreatment of blood pressure. Lisinopril does not appear to be bound to other serum proteins. Lisinopril does not undergo metabolism and is excreted unchanged entirely in the urine. It is used in the treatment of hypertension and heart failure in prophylactic treatment after myocardial infarction and in diabetic nephropathy. Lisinopril works by causing blood vessels relax, lowering blood pressure and increasing the supply of blood and oxygen to the heart. For this reason it is also sometimes used, alongside other medicines, to treat heart failure and circulatory problems associated with diabetics.
Labetalol hydrochloride is chemically known as 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino) ethyl salicylamide hydrochloride. Labetalol belongs to the group of medicines called β-blockers; it is the first adrenergic antagonist capable of blocking both α and β receptors in the heart, lungs and other organs of the body. There are 4 stereoisomers of labetalol, each of which has a distinct profile of action of subtypes of α and β receptors. Labetalol is 5 times more potent in blocking β than α receptors. These are often referred to as the ‘fight or flight’ chemicals as they are responsible for the body’s reaction to stressful situations. Labetalol is used to treat high blood pressure and also to cause controlled low blood pressure during anaesthesia. Beside these important pharmacological activities, labetalol therapy exhibits hepatotoxicity and renal failure due to overdosage. Fall in blood pressure (both systolic and diastolic) is due to α1 and β1 blockade as well as β2 agonism (vasodilation). Relatively high doses reduce both cardiac output and total peripheral resistance.
Perindopril erbumine is chemically, tertiary-butyl-amine salt of \([\text{2S-1-(R, R)}\ 2\alpha, 3\alpha\beta, 7\alpha\beta]-1-[2-(1\text{-ethoxy carbonyl butyl}] \text{amino}-\text{oxopropyl, octahydo-1H indole-2 carboxylic acid. It is the first member of a new chemical class of a non-peptide angiotensin II receptor antagonist used in the treatment of hypertension and congestive heart failure.}

S-Perindopril is a pro-drug that is hydrolyzed to the active metabolite perindoprilat. The members of this class have the common property of acting as pro-drugs, being converted to the active diacid through the metabolic process by liver and intestinal enzymes. Its ability to lower blood pressure is comparable to, or better than that of other antihypertensive drugs. Recent studies have also proved that S-Perindopril suppresses the tumour growth and angiogenesis and can modify clinical features of Parkinson’s disease. R-Perindopril, which is a by-product produced in the synthesis of S-Perindopril, does not have the same activity as the S-enantiomer.
References


Table 1.1: Major Challenges for Manufacturing and Control

- Maintain inventory for development and marketing
- Physical and chemical characterization
- Develop and validate robust analytical methods
- Scale-up manufacturing process
- Optimize and validate process and controls
- Minimize cost of goods
- Formulation development
- Select/validate appropriate container/closure system
- Set appropriate control and release specifications
- Define expiration dating (stability/compatibility)
- Design label and outer packaging
- Provide for adequate warehousing and distribution
Table 1.2: Chemistry, Manufacturing and Controls Testing Conducted in the Drug Development Pathway

### Discovery to Phase I
- Active pharmaceutical ingredient (API) solubility studies
- API identification by spectroscopic analysis
- Chromatographic purity
- pKa determination
- Moisture determination
- Partitioning studies
- Characterization studies
- Development of chromatographic analysis of API
- Residual solvents identification and quantitation
- Short-term stability of API
- Reference standard certification

### Phase I
- Validation of analytical methods for API and Phase I product
- Stability of Phase I product
- Release of Phase I clinical test materials

### Phases II and III
- Continued analytical method refinements and validations as per product and process improvements
- Release of Phase II and III clinical test materials
- Release of product for long-term toxicology studies
- Long-term stability program
- Cleaning validation support
- Analysis of manufacturing process validation samples
- Container/closure extractable/leachable studies

### Post Approval
- QC release of product
- QC release of API
- QC release of excipients
- Post approval stability programs
- Scale-up and post approval changes
Table 1.3: Components of Stability Testing Protocol

- Technical grade and manufacturer of drug substance and excipients
- Type, size, and number of batches
- Type, size, and source of containers and closures
- Test parameters
- Test methods
- Acceptance criteria
- Test time points
- Test storage conditions
- Container storage orientations
- Sampling plan
- Statistical analysis approaches and evaluations
- Data presentation
- Retest or expiration dating period
- Stability commitment
Define performance specifications

Devise development experiments

Execute and evaluate results

Plan method validation experiments

Calibration model, range and linearity

Precision and accuracy

Collate results

Write validation report

Apply validated method

Fig.1.2: The process of development, validation and routine use of an analytical method.
Table 1.4: Assay of drugs in pharmaceutical formulation by UV-visible spectrophotometric procedures.

<table>
<thead>
<tr>
<th>Name of drugs</th>
<th>Reagents used</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>$m$-cresol</td>
<td>640</td>
<td>64</td>
</tr>
<tr>
<td>Amiodarone HCl</td>
<td>$p$-chloranilic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone</td>
<td>535, 575</td>
<td>65</td>
</tr>
<tr>
<td>Amlodipine besylate</td>
<td>$p$-chloranilic acid, ninhydrin in DMF medium, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, ascorbic acid, 1, 10-phenanthroline, 2, 2'-bipyridyl, ammonium heptamolybdate</td>
<td>540</td>
<td>66</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>potassium iodate, folin-ciocalteau phenol reagent</td>
<td>520</td>
<td>70</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>potassium iodate, folin-ciocalteau phenol reagent</td>
<td>520</td>
<td>70</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1-chloro-2,4-dinitrobenzene</td>
<td>380</td>
<td>72</td>
</tr>
<tr>
<td>Benidipine HCl</td>
<td>methanol</td>
<td>238</td>
<td>73</td>
</tr>
<tr>
<td>Captopril</td>
<td>potassium iodate</td>
<td>510</td>
<td>74</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>folin-ciocalteau phenol reagent, tris buffer</td>
<td>750</td>
<td>71</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>sodium metavanadate, bromophenol blue, bromothymol blue, bromocresol green</td>
<td>750</td>
<td>71</td>
</tr>
<tr>
<td>Diltiazem HCl</td>
<td>KMnO$_4$ in alkaline medium</td>
<td>610</td>
<td>78</td>
</tr>
<tr>
<td>Famotidine</td>
<td>ninhydrin</td>
<td>590</td>
<td>79</td>
</tr>
<tr>
<td>Flunarizine dihydrochloride</td>
<td>iodine</td>
<td>295, 355</td>
<td>80</td>
</tr>
<tr>
<td>Labetalol hydrochloride</td>
<td>sodium nitroprusside</td>
<td>695</td>
<td>81</td>
</tr>
<tr>
<td>Levodopa</td>
<td>Ce (IV) nitrate in H$_2$SO$_4$ medium, NaOH</td>
<td>510</td>
<td>82</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>Ninhydrin, ascorbic acid</td>
<td>690</td>
<td>83</td>
</tr>
<tr>
<td>Menadione</td>
<td>NaOH in presence of amine, ethyl acetoacetate and ethanolic ammonia</td>
<td>450</td>
<td>84</td>
</tr>
<tr>
<td>Methyldopa</td>
<td>Ce (IV) nitrate in H$_2$SO$_4$ medium</td>
<td>550</td>
<td>82</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>Ninhydrin in DMF, KMnO$_4$ in alkaline medium</td>
<td>595</td>
<td>86</td>
</tr>
<tr>
<td>Mometasone</td>
<td>methanol</td>
<td>248</td>
<td>88</td>
</tr>
<tr>
<td>Drug</td>
<td>Reaction/Reagents</td>
<td>λ (nm)</td>
<td>E (ε)</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------------------------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>furoate</td>
<td>Nalidixic acid persulfate in alkaline medium</td>
<td>320, 390</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Nicorandil brucine-sulfanilic acid in H$_2$SO$_4$ medium</td>
<td>410</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3-methyl-2-benzothiazolone hydrazone hydrochloride and metol sulfuric acid in H$_2$SO$_4$ medium</td>
<td>560</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3-methyl-2-benzothiazolone hydrazone hydrochloride and DL-3,4-dihydroxyphenylalanine</td>
<td>530</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Nifedipine KMnO$_4$ in neutral medium</td>
<td>530</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>4-methyl amino phenol and K$_2$Cr$_2$O$_7$</td>
<td>525</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>bromocresol green</td>
<td>415</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>bromophenol blue</td>
<td>415</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>bromothymol blue</td>
<td>415</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>eriochrome black-T</td>
<td>520</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>KOH in dimethylsulfoxide</td>
<td>430</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin KMnO$_4$ in alkaline medium</td>
<td>603</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin bromocresol purple</td>
<td>408</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>bromocresol blue</td>
<td>414</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pantoprazole 1-flouro-2,4-dinitrobenzene</td>
<td>420</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Ramipril potassium iodide and potassium iodate</td>
<td>352</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Roxatidine acetate hydrochloride</td>
<td>530</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2,3-dichloro-5,6-dicyano-1,4-benzoquinone</td>
<td>530</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3-methyl-2-benzothiazolone hydrazone hydrochloride and potassium persulfate</td>
<td>530</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>Silymarin KMnO$_4$ in neutral medium</td>
<td>530</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>3-methyl-2-benzothiazolone hydrazone hydrochloride and potassium persulfate</td>
<td>430</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim persulfate in alkaline medium</td>
<td>355</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Thyroxine nitrous acid</td>
<td>420</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>Verapamil chloramine-T</td>
<td>425</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>N-bromosuccinimide</td>
<td>415</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>potassium metaperiodate in H$_2$SO$_4$ medium</td>
<td>425</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>tropaeolin 000 No.1 at pH 4.0</td>
<td>400</td>
<td>107</td>
</tr>
</tbody>
</table>
### Table 1.5: Validation of analytical methods International definitions.

<table>
<thead>
<tr>
<th>Organisation</th>
<th>Applicability</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC</td>
<td>World wide</td>
<td></td>
</tr>
<tr>
<td>ILAC</td>
<td>World wide</td>
<td></td>
</tr>
<tr>
<td>WELAC</td>
<td>Europe</td>
<td></td>
</tr>
<tr>
<td>ICH</td>
<td>Europe, Japan, USA</td>
<td>Only pharmaceutical products</td>
</tr>
<tr>
<td>ISO</td>
<td>World wide</td>
<td>Lack definition of selectivity and specificity</td>
</tr>
</tbody>
</table>

**Abbreviations:**

- **ILAC**, International Laboratory Accreditation Conference.
- **WELAC**, Western European Laboratory Accreditation Co-operation
- **ICH**, International Conference on Harmonization.
- **ISO**, International Organization for Standardization.
<table>
<thead>
<tr>
<th><strong>Table 1.6: Quantitative approaches to demonstrate accuracy according to ICH.</strong></th>
</tr>
</thead>
</table>
| **Drug Substance** | Application of the analytical procedure to a reference material.  
Comparison of the results with those of a second, well characterised procedure. |
| **Drug Product** | Application of the analytical procedure to synthetic mixtures of drug product components.  
Spiking of analyte to drug product.  
Comparison of the results with those of a second, well characterised procedure. |
| **Impurities (Quantitative)** | Spiking of the impurity to drug substances or product.  
Comparison of the results with those of a second, well characterised procedure. |
Table 1.7: Approaches for determining the detection and quantitation limits [167].

<table>
<thead>
<tr>
<th>Approach</th>
<th>Detection limit</th>
<th>Quantitation limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual evolution</td>
<td>Minimum level detection</td>
<td>Minimum level quantifiable</td>
</tr>
<tr>
<td>Signal-to-noise</td>
<td>3:1 or 2:1</td>
<td>10:1</td>
</tr>
<tr>
<td>Standard deviation of the</td>
<td>3.3 × (S₀)/b</td>
<td>10.0 × (S₀)/b</td>
</tr>
<tr>
<td>response (S₀) b and the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>slope(b)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Verification with suitable number of samples.

\(^b\) Standard deviation of the blank, or standard deviation of the calibration line.
Fig. 1.3: Useful range of an analytical method: LOQ = Limit of quantitative measurement and LOL = Limit of linear response