1. Introduction...
1. INTRODUCTION

Quality control plays a central role in determining the safety and efficacy of medicines. Modern medicines for human use are required to meet exacting standards which relate to their quality, safety and efficacy. The evaluation of safety and efficacy and their maintenance in practice is dependant upon the existence of adequate methods for quality control of the product. Highly specific and sensitive analytical techniques hold the key to the designing, development, standardization and quality control of medicinal products.

Modern pharmaceutical formulations are complex mixtures including, active ingredients, and a number of inert materials such as diluents, disintegrants, colors and flavors. In order to ensure quality and stability of final product these mixtures must be separated and quantified. Modern analytical techniques like spectroscopic methods, chromatographic methods, electrometric methods and thermometric methods are extremely sensitive providing precise and detailed information from small samples of material. They are rapidly applied, and in general are readily amenable to automation. For these reason they are now in widespread use in product development, in the control of manufacture and formulation as a check on stability during storage, and in monitoring the use of drugs and medicines.

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

The objective of analytical method is to generate reliable and accurate data regardless of whether it is for acceptance of raw materials, release of the drug substances and products, in-process testing for quality assurance, stability studies or establishment of the expiration dating period. Data are generated for the qualitative and quantitative testing during development and post approval of the drug products.

The development of analytical method begins with the selection of analytical principle. The information required for the selection of analytical method includes nature of the sample, degree of separation required, analytical procedures available, amount of samples available and degree of accuracy and precision required. After selecting an appropriate analytical principle, method development and validation procedure starts. The purpose of this process is to confirm the viability of the method chosen and to show that the procedure is sufficiently analytically robust to allow preliminary validation to be carried out.
Introduction

As per FDA guideline, assay analytical method for stability studies should be stability indicating. Stability indicating method is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties (e.g., active ingredient) of the drug substance and drug product. A stability-indicating assay accurately measures the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. Stability indicating method can be developed by performing stress testing on drug substance and demonstrating that the method is valid in presence of degradation products produced under stress conditions. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures. The testing should include the effect of temperature, humidity, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension.

Analytical method validation

Analytical method validation is the process by which it is established, by laboratory studies, that the performance characteristic of the method meet the requirements for the intended analytical applications. The objective of the analytical method validation is to demonstrate that it is suitable for its intended purpose. Following analytical procedures requires to be validated

- Identification tests
- Dissolution test
- Limit test for the control of impurities
- Quantitative tests of the active moiety in samples of drug substances.

Typical analytical performance characteristics that should be considered in the validation of the analytical methods are as below.

Specificity

Specificity is the ability to assess the analyte in the presence of components which may be expected to be present which includes impurities, degradants, matrix etc. In case of assay, specificity is assessed by demonstrating that the procedure is unaffected by the presence of impurities or excipients. It is carried out by spiking the
drug substance with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials. If impurities or degradation products are unavailable specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well characterized procedure. These comparisons should include samples stored under relevant stress conditions. ICH states that when chromatographic procedures are used, representative chromatogram should be presented to demonstrate degree of selectivity.

Accuracy
The accuracy of an analytical procedure is the closeness of the test result obtained by that method to the true value. Accuracy should be established across its range. In case of assay of a drug in formulated product, accuracy may be determined by application of analytical method to synthetic mixture of the drug product components to which known amount of analyte have been added with in the range of the method. If it is not possible than it is acceptable to add known quantities of analyte to drug product or to compare results with those of a second well characterized method the accuracy of which has been defined. Accuracy is calculated as % recovery by the assay of the known added amount of analyte in the sample or as difference between the mean and the accepted true value. ICH states that the accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering specified range.

Precision
The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment. Repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure or a minimum of 6 determinations at 100% of the test concentrations. Intermediate precision refers to within laboratory variation, as on
different days, or with different analysts or equipment within the same laboratory. Reproducibility is assessed by means of an inter-laboratory trial.

Detection limit
The detection limit is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Several approaches including visual evaluation, signal to noise approach (3:1) and standard deviation of slope and response are used to determine the detection limit. Using standard deviation of the response and the slope approach, detection limit may be expressed as

\[ \text{LOD} = \frac{3.3\sigma}{S} \]

Where \( \sigma \) is the standard deviation of y-intercept of regression line and \( S \) is the slope of the calibration curve.

Quantitation limit
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Several approaches including visual evaluation, signal to noise approach (10:1) and standard deviation of slope and response are used to determine the quantitation limit. Using standard deviation of the response and the slope approach, quantitation limit may be expressed as

\[ \text{LOQ} = \frac{10\sigma}{S} \]

where \( \sigma \) is the standard deviation of y-intercept of regression line and \( S \) is the slope of the calibration curve.

Linearity and Range
The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Linearity should be established across the range of the analytical procedure. The data like correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be reported to demonstrate linearity. For the establishment of linearity, a minimum of 5 concentrations should be studied.
Introduction

As per ICH following minimum specified ranges should be considered:

- For the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;
- For content uniformity, covering a minimum of 70 to 130 percent of the test concentration.
- for dissolution testing: +/- 20 % over the specified range

Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during its normal usage. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

Ruggedness
The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analyst, different instruments, different lots of reagents, different days etc. The ruggedness of the analytical method is determined by analysis of aliquots from homogeneous lots in different laboratories, by different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay.

Sample solution stability
Solution stability of the drug substance or drug product after preparation should be evaluated according to the test method. To generate reproducible and reliable results the samples, standards and reagents used for the method must be stable for a reasonable time. Generally 24 h stability is desired for solutions and reagents that need to be prepared for each analysis.

System suitability
System suitability parameters are established to ensure that the validity of the analytical method is maintained whenever used. System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that
the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. USP defines parameters that can be used to determine system suitability prior to analysis. These parameters include theoretical plate number, tailing factor, capacity factor (k’), relative retention, resolution and relative standard deviation of peak area for repetitive injection.