1. INTRODUCTION

1.1 DIABETES

346 million people worldwide have diabetes worldwide. More than 80% of diabetes deaths occur in low- and middle-income countries. Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type 2 diabetes. Over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). People with type 1 diabetes require insulin; people with type 2 diabetes can be treated with oral medication, but may also require insulin. Diabetes treatment is aimed at keeping blood glucose levels as close to the normal range as safely possible. Studies have shown this reduces the risk of developing major complications associated with type 1 and type 2 diabetes.

1.2 DEVELOPMENT OF NEW ANALYTICAL METHODS

A regulatory analytical procedure is the analytical procedure used to evaluate a defined characteristic of the drug substance or drug product. An alternative analytical procedure is an analytical procedure proposed by the applicant for use instead of the regulatory analytical procedure. Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide 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, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug, and degradants generated, during the stability study.

The modern methods of choice for quantitative analysis are HPLC, GC, and HPTLC, which are highly sophisticated. Chromatographic methods are commonly used in regulatory laboratories for the qualitative and quantitative analysis of drug substances, drug products, raw materials and biological samples throughout all phases of drug development, from research to quality control. High performance liquid chromatography (HPLC) is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. The rapid growth of HPLC has been
facilitated by the development of reliable, moderately priced instrumentation and efficient columns. High performance thin-layer chromatography (HPTLC) is a classical separative technique that has enjoyed wide spread popularity particularly in the analysis of complex mixtures of natural origin. Now-a-days HPLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput, and need for minimum sample clean-up. The major advantage of HPLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

1.3 Validation of analytical methods

As defined by the USP, method validation provides an assurance of reliability during normal use, and is sometime referred to as “the process of providing documented evidence that the method does what it is intended to do.” The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. To be fit for the intended purpose, the method must meet certain validation characteristics. Typical validation characteristics, which should be considered, are: selectivity (specificity), linearity, range, accuracy, precision, and limit of detection, limit of quantitation, ruggedness, robustness and system suitability testing.

1.3.1 Selectivity (Specificity)

Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture.

1.3.2 Linearity

The linearity is the ability of analytical procedure to produce test results which are proportional to the concentration (amount) of analyte in samples within a given concentration range, either directly or by means of a well-defined mathematical transformation. Linearity should be determined by using a minimum of six standards whose concentration span 80 – 120% of the expected concentration range. The results should not show a significant deviation from linearity, which is taken to mean that the correlation coefficient, $r > 0.99$, over the working range (80 –120%).
1.3.3 Range
The specified range is normally derived from the linearity studies. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical method has suitable levels of precision, accuracy and linearity.

1.3.4 Accuracy
The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways and the method should be appropriate to the matrix. The accuracy of an analytical method may be determined by:

(A) Analyzing a sample of known concentration and comparing the measured value to the „true” value. However, a well characterized sample (e.g., reference standard) must be used.

(B) Spiked – placebo (product matrix) recovery method:
In the spiked –placebo recovery method, a known amount of pure active constituent is added to formulation blank [sample that contains all other ingredients except the active (s)], the resulting mixture is assayed, and the results obtained are compared with the expected result.

(C) Standard addition method:
In the standard addition method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer.
In both methods (spiked – placebo recovery and standard addition method), recovery is defined as the ratio of the observed result to the expected result expressed as a percentage.
% Recovery calculated by formula:
\[ \frac{N \sum xy - (\sum x)(\sum y)}{N \sum x^2 - (\sum x)^2} \times 100 \]

N = Number of observations.
Y = Amount of drug found.
X = Amount of standard drug added.

1.3.5 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

(a) Repeatability:
Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

(b) Intermediate Precision:
Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

(c) Reproducibility:
Reproducibility expresses the precision between laboratories.
For these guidelines, a simple assessment of repeatability will be acceptable. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. A minimum of 5 replicate sample determinations should be made together with a simple statistical assessment of the results, including the percent relative standard deviation.

The standard deviation (SD) is calculated from the following formula:
\[ SD = \frac{\sum (X_i - X)^2}{N-1} \]
Xi = individual measurement in a set
X = arithmetic mean of the set and
N = total number of replicated measurements taken in the set.

Precision between different samples can be compared with relative standard deviation (RSD) as follows.

\[ \text{RSD} = \frac{S}{X} \]

% RSD or coefficient of variance (CV) = \( \text{(S/X)} \times 100 \)

1.3.6 Limit of Detection (LOD)

The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value. The lowest calibration standard which produces a peak response corresponding to the analyte should be measured \( n \) times (normally 6-10). The average response (X) and the standard deviation (SD) calculated by formula;

\[ \text{LOD} = X + (3 \times SD). \]

1.3.7 Limit of Quantitation (LOQ)

The limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. LOQ can be calculated by formula;

\[ \text{LOQ} = X + (10 \times SD). \]

1.3.8 Ruggedness

Ruggedness is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days etc.
1.3.9 Robustness
It is the measure of capability of analytical method to remain unaffected by small but deliberate variation in the method parameters and provides an indication of its reliability during normal range. Robustness testing is normally restricted to methods that are to be used repetitively in the same laboratory.

1.3.10 System suitability testing
System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. For high performance liquid chromatographic analysis, system suitability tests are

**Number of theoretical plates (N)**, is a measure of column efficiency. It is calculated by formula

\[ N = 16 \left( \frac{t}{w} \right)^2 \text{ or } N = 5.54 \left( \frac{t}{wh/2} \right)^2 \]

* t = Retention time of the substance.
* w = Width of peak measured by extrapolating the relatively straight sides to the base line for respective substances.
* wh/2 = Peak width at half height.

**Resolution (R)** is a function of column efficiency and is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug. Resolution can be calculated by formula;

\[ R = 2 \frac{(t_2-t_1)}{w_1+w_2} \]

* t1, t2 = Retention of respective substances
* w1, w2= Width of peak measured by extrapolating the relatively straight sides to the base line for respective substances.
**Relative standard deviation (RSD)**, calculated from data of five replicate injections of analyte, if the requirement is less than 2.0% or less; data from six replicate injections are used if RSD requirement is more than 2.0%.

**The tailing factor (T)**, measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increase as tailing becomes more pronounced. In some cases, value less than unity may be observed. As peak asymmetry increases, integration, and hence precision, becomes less reliable. Tailing factor calculated by formula;

\[ T = \frac{w_{0.05}}{2f} \]

\( w_{0.05} = \) Width of peak at 5% height.

\( f = \) distance between the perpendicular dropped from the peak Maximum and the leading edge of the peak at 5% height