CHAPTER 1

INTRODUCTION
Section 1.0

GENERAL INTRODUCTION TO DIABETES, PHARMACEUTICALS, PHARMACEUTICAL ANALYSIS, OBJECTIVES AND SCOPE

DIABETES

The term **diabetes mellitus** describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. Diabetes was first recognized around 1500 BC by the ancient Egyptians, who considered it a rare condition in which a person urinated excessively and lost weight. The term diabetes mellitus, reflecting the fact that the urine of those affected had a sweet taste, was first used by the Greek physician Aretaeus. It was not until 1776, however, that Matthew Dobson actually measured the concentration of glucose in the urine of such patients and found it to be increased [2]. In 1889, Joseph von Mering and Oskar Minkowski found that removing the pancreas from dogs resulted in fatal diabetes, providing the first clue that the pancreas plays a key role in regulating glucose concentrations [3,4]. In 1910, Edward Albert Sharpey-Schafer hypothesized that diabetes was due to the deficiency of a single chemical produced by the pancreas; he called this chemical insulin, from the Latin word *insula*, meaning island and referring to the pancreatic islet cells of Langerhans. In 1921, Frederick Banting and Charles Best actually discovered insulin when they reversed diabetes that had been induced in dogs with an extract from the pancreatic islet cells of healthy dogs [5,6]. Together with James Collip and John Macleod, they purified the hormone insulin from bovine pancreases and were the first to use it to treat a patient with diabetes. Banting and Macleod were awarded with Nobel prize for their invention in the year 1923. The production of insulin and its therapeutic use quickly spread around the world. This series of events may be the most dramatic example of the rapid translation of a discovery in basic science into a benefit for patients. Once insulin injections became available, young people with insulin deficiency who had previously faced almost certain, painful death within weeks to months were able to survive for prolonged periods of time [7].

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.
In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. According to Wild et al. [11] the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease [10,11]. India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country. Many influences affect the prevalence of disease throughout a country, and identification of those factors is necessary to facilitate change when facing health challenges [12, 13].

**Diabetes origin in body**

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading
to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes [8].

Types of diabetes

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 [15] and, in modified form, in 1985 [16]. The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM (Insulin Dependent Diabetes Mellitus) or Type 1, and NIDDM (Non Insulin Dependent Diabetes Mellitus) or Type 2. In the 1985 Study Group Report the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition-related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports other classes of diabetes included Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). These were reflected in the subsequent International
Nomenclature of Diseases (IND) in 1991, and the tenth revision of the International Classification of Diseases (ICD-10) in 1992. The 1985 classification was widely accepted and is used internationally. It represented a compromise between clinical and aetiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or aetiology was unknown. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complementary aetiological classification [1].

In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response [8]. The third category of diabetes is Gestational diabetes, hyperglycaemia with blood glucose values above normal but below those diagnostic of diabetes, occurring during pregnancy. Women with gestational diabetes are at an increased risk of complications during pregnancy and at delivery. They are also at increased risk of type 2 diabetes in the future [17].

**Classification of Diabetes mellitus**

<table>
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<th>Class name</th>
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<td><strong>Type-1:</strong> Insulin-dependent diabetes mellitus (IDDM)</td>
<td>Low or absent levels of circulating endogenous insulin and dependent on injected insulin to prevent ketosis and sustain life&lt;br&gt;Onset predominantly in youth but can occur at any age&lt;br&gt;Associated with certain HLA and GAD antigens&lt;br&gt;Abnormal immune response and islet cell antibodies are frequently present at diagnosis&lt;br&gt;Etiology probably only partially genetic, as only ~35% of monozygotic twins are concordant for IDDM</td>
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<td><strong>Type-2:</strong> Non-insulin-dependent diabetes mellitus (NIDDM)</td>
<td>Insulin levels may be normal, elevated, or depressed; hyperinsulinemia and insulin resistance characterize most patients; insulinopenia may develop as the disease progresses&lt;br&gt;Not insulin-dependent or ketosis-prone under normal circumstances, but may use insulin for treatment of hyperglycaemia&lt;br&gt;Onset predominantly after age 40 years but can occur</td>
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approximately 50% of men and 70% of women are obese
Etiology probably strongly genetic as 60%-90% of monozygotic twins are concordant for NIDDM

| Gestational diabetes (GDM) | Glucose intolerance that has its onset or recognition during pregnancy
Associated with older age, obesity, family history of diabetes
Conveys increased risk for the woman for subsequent progression to NIDDM
Associated with increased risk of macrosomia |

| Other types of diabetes, including diabetes secondary to or associated with: Pancreatic disease Hormonal disease Drug or chemical exposure Insulin receptor abnormalities Certain genetic syndromes | In addition to the presence of the specific condition, hyperglycemia at a level diagnostic of diabetes is also present
Causes of hyperglycemia are known for some conditions, e.g., pancreatic disease; in other cases an etiologic relationship between diabetes and the other condition is suspected |

Prevention

Simple lifestyle measures have been shown to be effective in preventing or delaying the onset of type 2 diabetes. To help prevent type 2 diabetes and its complications, people should:

- achieve and maintain healthy body weight;
- be physically active – at least 30 minutes of regular, moderate-intensity activity on most days. More activity is required for weight control;
- eat a healthy diet of between 3 and 5 servings of fruit and vegetables a day and reduce sugar and saturated fats intake;
- avoid tobacco use – smoking increases the risk of cardiovascular diseases.

DIAGNOSIS AND TREATMENT

Diabetes cannot be cured, so treatment aims to keep the blood glucose levels as normal as possible and to control the symptoms, to prevent the diseases developing later stages of life. Early diagnosis can be accomplished through relatively inexpensive blood testing.
The aim of the treatment is primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and, by eliminating various risk factors, to increase longevity [18].

Treatment of diabetes involves lowering blood glucose and the levels of other known risk factors that damage blood vessels. Tobacco use cessation is also important to avoid complications. Bastaki [19] in his review article collected the data on treatment of Diabetes Mellitus as below;

**Non-pharmacological interventions**

**Life style**

Primary prevention is the main aim at preventing diabetes from occurring in susceptible individuals or in general population. Regular physical activity is an important component of the prevention and management of type 2 diabetes mellitus. By tradition most of the recommendations for people with diabetes were low carbohydrate diets. More emphasis were placed on the use of complex carbohydrates or starches and the avoidance of simple carbohydrates or sugars on the belief that simple sugars would be digested and absorbed much more quickly. Ranges of non-nutritive sweeteners (including saccharin, aspartame, cyclamate, acesulphame K) are available for diabetics and may be useful if added to drinks and cooking. Though aspartame is a dipeptide, it is intensely sweet and very little quantities are required to make food and drinks palatable. All these sweeteners provide a useful means of reducing energy intake. Evidence suggests that reducing protein intake to the levels recommended by WHO (0.6 g/kg/day as a safe intake) can reduce albuminuria and improve renal hemodynamics in type 1 diabetes patients with incipient and established nephropathy [20,21]. Such diets do not worsen blood glucose levels [22] and protein undernutrition does not occur in the long term [23].

**Pharmacological interventions**

**Herbal treatment of diabetes**

Rahmann and Zaman [24] classified more than 100 medicinal plants for the treatment of type-2 diabetes. This review has classified the plants according to their botanical name, country of origin, parts used and nature of active agents. One such plant is Momordica charantia (Linn/Family: Cucurbaceae) whose fruit
is known as Karela/corilla, or bitter gourd. This plant is commonly cultivated in India, China, East Africa and Central and South America. Several studies have examined the antidiabetic potential of bittergourd, both in humans as well as in animals. The first clinical studies into the effect of fresh juice of bittergourd on management of diabetes was by Akhtar [25]. This study suggested that administration of the fresh juices of bittergourd could treat all symptoms of diabetes including polyurea, polydipsia and polyphagia. Urinary excretion of sugar was also reduced and insulin injections were stopped.

**Insulin treatment**

It is an accepted fact that insulin is the most potent glucose-lowering agent, with hypoglycaemia being the only major dose-limiting factor. The introduction of insulin to treat diabetes has saved an estimated 5 million years of life for patients with type 1 diabetes during the year 2000 [26].

**Oral hypoglycemic treatment**

These are the group of drugs that may be taken singly or in combination to lower the blood glucose in type 2 diabetes. Type 2 diabetes can be due to increased peripheral resistance to insulin or to reduced secretion of insulin. They should be used together with changes in diet and lifestyle to achieve good glycaemic control, and it is customary to monitor such changes for three months before considering medication. Most of the oral antihyperglycemic agents can be administrated individually or combined with each other is much effective.

**PHARMACEUTICALS**

Pharmaceutical drug is a substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease. It is also referred to as medicine, medication or medicament, can be defined as any chemical substance intended for use in the medical diagnosis, cure, treatment, or prevention of disease [27,28]. A drug is any substance (except food and water) which, when taken into the body, alters the body's function either physically and/or psychologically. Pharmaceutical drugs may be used for a limited span, or on a regular basis for chronic disorders. There are different classes of drugs which will have different function on the body. In the ancient times, a large part of medicinal products used were natural
products mostly derived from plants but in subsequent centuries it was agreed that such products must be pure for effective utilization as pharmaceutical products.

Pharmaceutical drug analysis provides information on the identity, purity, content and stability of starting materials, excipients, active pharmaceutical ingredients (APIs) and also the secondary pharmaceutical product(s) \( i.e., \) the dosage forms having either single or multi-component formulated product \[29\]. The quality of the drug product may deviate from the standard required but in carrying out an analysis one can confirm that whether the quality of the product is of the required standard or not \[30\]. The fundamental reasons for the ‘analysis of drug substances’ are perhaps due to the tremendous growth in the progress of ‘medicinal chemistry’ towards achieving one ultimate objective which is to obtain \textbf{‘better drugs for a better world’} \[31\].

The drug development process involves a number of activities which are carried out simultaneously, as shown by the oversimplified depiction in \textbf{Fig. 1}. Once a molecule is discovered that has desirable biological activity, the process of creating a pharmaceutical drug product from this molecule begins. As toxicology and efficacy studies are undertaken, methods for manufacture of the active molecule and for its delivery in therapeutic doses are sought. Critical to the latter effort is finding a form of the active molecule which exhibits appropriate physical properties. The form ultimately selected, called the active pharmaceutical ingredient (API), or drug substance, must be stable and bioavailable enough to be formulated into a drug product, such as a tablet or suspension. This formulation must be effective at delivering the active molecule to the targeted biosystem \[32\].
Regulators worldwide require increasingly high quality and safety standards from the pharmaceutical industry. To ensure these standards are met, reliable analysis tools and methods are constantly required and developed by analytical scientists. Pharmaceutical analysis therefore plays a pivotal role in advancing the concepts and theories of analytical science, as well as providing important information on practical aspects of drug design, quality control, and quality assurance of industrial manufacturing. Pharmaceutical analysis methods are traditionally and commonly applied to the chemical analysis of drug molecules. However, in the last two decades, modern pharmaceutical analysis has evolved enormously, capitalizing on combination techniques, high-throughput technologies, chemometrics, and most recently miniaturization and nanotechnology. The combination of various techniques allows the modern pharmaceutical analyst to exploit the virtues of each technique and, in turn, to improve the overall quality of analysis. Indeed, modern analytical techniques and methods offer the possibility of increasing the amount of information received from individual analysis, with reduced cost, analysis time, and sample volumes [33].
The purpose of analytical method

An analytical method details the steps necessary to perform an analysis. The use of analytical method during the development and manufacturing provides the following information.

a. Potency, which can relate directly to the requirement of a known drug.

b. Impurities, which will relate with the safety of the drug.

c. Key characteristic form such as crystallinity, drug release etc.

d. Degradation products, to confirm the method is stability-indicating.

METHOD DEVELOPMENT

Pharmaceutical analysis plays a very prominent role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. Rapid increase in pharmaceutical industries and production of drug in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries. As a consequence, analytic method development became the key element of any pharmaceutical drug development program. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Recent development in analytical methods has been resulted from the advancement of analytical instruments. The improvement of the analytical method development and analytical instruments have reduced the time of analysis, increased precision and accuracy and reduced costs of analysis. As a consequence, most of pharmaceutical organizations are investing huge amount of money for the establishment of advanced analytical laboratories. Analytical techniques are developed and validated for active pharmaceutical ingredients (API), excipients, drug products, degradation products and related substances, residual solvents, etc. As a result, it has become an integral part of the requirements of the regulatory organization. Analytical method development finally results in official test methods. These methods are used in quality control laboratories to ensure the identity, purity, safety, efficacy and performance of drug products. Regulatory authorities are placing greater emphasis on analytical methods in manufacturing. Drug approval by regulatory authorities requires the
applicant to prove control of the entire process of drug development by using validated analytical methods [34]. The following six ‘M’ s are the prerequisites for analytical method development

![Diagram of Six “M”s]

**Pharmaceutical analysis methods**

HPLC-UV, HPLC-NMR, HPLC-NMR-MS, GC-MS, LC-MS/MS, UPLC, FL-spectrofluorimetry, phosphorimetry, supercritical fluid chromatography (SFC)-UV, electro-analytical, etc. [26] are the recent techniques used in pharmaceutical analysis. It is true that these instrumental approaches make possible a great many things that could not be accomplished previously using conventional techniques. They also make possible, in some cases, faster analysis than was possible with the chemical methods. Although modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are from traditional techniques many of which are still used today.

There are many reasons for the persistence of the chemical types of analysis, can be summarized as follows:

There are many chemical situations which are better handled by chemical, rather than instrumental methods. The broad spectrum of reactions available gives the analyst quite versatility. For e.g., we find that the analysis of complex systems relies heavily on conventional chemical analysis, since specific reactions are generally available for classes of organic compounds of pharmaceutical importance. In addition, the area of trace analysis relies heavily on chemical methods to develop specific colors for the materials in question.
Another advantage of the conventional chemical analysis, particularly titrimetry, can be stated as follows: Most instrumental analyses are dependent on calibration curves which require pure samples of the compounds in question. Titrimetry does not require such calibrations. Titration methods are still widely used in the analysis for the assay of bulk-drug materials. Its share in the European Pharmacopoeia is almost 70%, and more than 40% in United States Pharmacopoeia (USP) for the determination of low molecular weight organic compounds [35]. Hence, this approach is the most practical when the analytical laboratory is faced with problem of pure samples. The cost of equipment of conventional analysis is generally quite low [36].

Taking into consideration the technical and economical constraints, it is necessary that the recommended methods should permit their use by pharmaceutical industries located in the developing/under developed countries. It is significant to stress that even simple techniques like titrimetry and spectrophotometry result in sensitive and accurate measurements with clear advantages of speed, simplicity, cost-effectiveness and zero/or easy maintenance. The large volume of literature devoted to their application in almost every field of scientific research constitutes irrefutable evidence of their utility. It is, therefore, reasonable to assume that the analytical procedures involving the use of such simple techniques will find wider applications in the field of pharmaceutical analysis as well.

Furthermore, majority of the methods currently in use in pharmaceutical analysis, like HPLC, LC-MS, TLC, HPTLC, GC, GC-MS, capillary-electrophoresis, etc., are purely physical methods. The use of purely physical methods in quantitative analysis in recent years has given rise to a concern among the academicians that there is a negligence of basic chemistry in analytical chemistry [37].

**Quality by Design (QbD)**

Quality by Design (QbD) is well established in the pharmaceutical industry for manufacturing processes ICH Q8 [38] for pharmaceutical development and ICH Q11 [39] for development and manufacture of drug substances). QbD is “A more systematic approach to development can include, for example, incorporation of prior knowledge, results of studies using design of
experiments, use of quality risk management, and use of knowledge management throughout the lifecycle of the product” [40]. The outcome of using QbD concepts is a well-understood product and process that consistently delivers its intended performance. The knowledge obtained during development may support the establishment of a design space and determines suitable process controls. These same QbD principles have been applied to the development of analytical methods, and are termed “Analytical QbD” (AQbD) [41-46].

OBJECTIVES AND SCOPE OF THE PRESENT STUDY

Treatment of type II diabetes is now possible with orally administrated hypoglycaemic agents that help to reduce blood sugar levels. Five major classes of chemically diverse hypoglycaemic drugs with different mechanisms of action have been developed for administration to patients. These are known as sulphonylureas (glipizide, glyburide, glimepiride, chlorpropamide and tolazamide), biguanides (metformin), thiozolidinones (pioglitazone and rosiglitazone), meglitides (repaglinide and nateglinide) and alpha-glucosidase inhibitors (acarbose and miglitol). Drawing one or two drugs from each of the above classes, the following eight drugs were selected for the present study.

1. Repaglinide (RPG)
2. Metformin hydrochloride (MFH)
3. Pioglitazone hydrochloride (PGH)
4. Nateglinide (NTG)
5. Glipizide (Olipazide) (GPZ)
6. Rosiglitazone (ROS)
7. Miglitol (MGL)
8. Chlorpropamide (CLP)

A careful survey of the analytical methods reported for the determination of the eight drugs in pharmaceuticals indicated that there existed a lot of scope for more and new methods for the determination.

The main goal of the present work was to develop various sensitive and selective analytical methods, employing easily available chemicals and cost effective techniques such as UV/Visible spectrophotometry and chromatography, for the assay of eight anti-diabetic drugs.
OBJECTIVE OF THE PRESENT STUDY

1. To develop simple, sensitive and cost-effective methods for the determination of eight antidiabetic drugs in pharmaceutical preparations using UPLC, HPLC, titrimetric and spectrophotometric techniques and to optimize the working conditions to maximize the performance characteristics.

2. To validate the developed methods for the linearity, LOD, LOQ, accuracy and precision, selectivity, robustness and ruggedness, according to the current ICH guidelines.

3. To apply the methods for bulk drug and commercial dosage forms. To study the interference from the commonly employed excipients by preparing placebo blank and synthetic mixture and to employ suitable solvents for extraction of interferer, if any.

4. As an attempt to expand the scope of application the developed methods, attempts will be made to apply the methods to determine the investigated drug in spiked human urine. This depends primarily on the sensitivity and selectivity of the developed method.

5. To evaluate the accuracy and precision of the methods developed by parallel analysis and by statistical evaluation of the results by applying Student’s t-test and F-test.

6. To study the accuracy of the developed methods by recovery study via standard-addition procedure.

7. To compare the developed methods with the existing methods with respect to performance characteristics.

The methods developed are a blend of chemical methods like titrimetry, visible spectrophotometry and physical methods such as UV-spectrophotometry, HPLC and UPLC. The latter techniques have been used for stability indicating studies in addition to assay.

In visible spectrophotometry, the reactions carried out were selected purely based on the availability of the functional groups in the drug under investigation. The presence of diverse functional groups and other oxidizing or reducing sites, in the drugs investigated offered reactions for their assay. Ion-pair
and charge-transfer complexation reactions were used as basis of methods that were developed for the studied drugs. In each case, based on the reaction stoichiometry between the drug and reactant or background literature, tentative reaction schemes have been proposed.

During method development, various experimental conditions like reaction time, reagent concentration, range of determination were studied and optimized. Spectrophotometric methods involved the evaluation of linear range, limits of detection and quantification, regression equation etc in addition to above studies. In HPLC and UPLC optimization of mobile phase composition and its pH, flow rate and detection mode etc, were optimized to achieve suitability and superiority of the methods.

In all the developed methods the quantification of drug was done only by relating the absorbance of the increasing/decreasing intensity of coloured species (reaction product formed between the drug and the reagents / unreacted reagent) (spectrophotometry) or response signal of the instrument with respect to the fixed parameters (in HPLC and UPLC). In spectrophotometry, the quantitation depends on the amount of radiation (light) absorbed by the analyte and which is described in Beer-Bouguer-Lambert’s law, commonly called Beer’s law. In chromatographic methods, quantification was done based on peak area obtained from the analyte of known or unknown concentration. The drug was quantified when it present both in pure form as well as in tablet form. The quantification was extended to spiked human urine sample wherever found feasible by successful elimination of the matrix substances and reporting percent recovery of the drug.

**METHOD VALIDATION**

Validation if defined as ‘finding or testing the truth of something’. When analytical methods are used to generate results about the characteristics of drug related samples it is vital that the results are trustworthy: they may be used as the basis for decisions related to administrating the drug to patients. A validation study is performed on an analytical method to ensure that reliable results are always obtained [37]. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) defines ‘The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose’ [47].
Validated analytical methods play a major role to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories. Analytical methods need to be validated, verified, or revalidated in the following instances [47-49]:

a. Before initial use in routine testing.

b. When transferred to another laboratory.

c. Whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or samples with a different matrix) and the change is outside the original scope of the method.

According to USP, analytical methods should be validated through laboratory tests: “Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications”. The required laboratory tests for method validation have been defined in different working groups of national and international committees and are described in the literature. Unfortunately, some of the definitions vary between the different organizations. Therefore, laboratories should have a glossary with definitions on their understanding of the terms. In an attempt to standardize, representatives from the industry and regulatory agencies from the United States, Europe and Japan defined parameters, requirements and methodology for analytical methods validation through the ICH. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated [48]. Typical validation characteristics which should be considered are listed below:

I. **Accuracy**: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

II. **Precision**: The precision of an analytical procedure expresses 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.
II a. **Repeatability:** Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

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

III. **Specificity:** Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

IV. **Detection Limit:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

V. **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. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

VI. **Linearity:** The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

VII. **Range:** The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

VIII. **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 normal usage.

The validation of the methods was done on pure drug solutions for intra-day and inter-day precision and accuracy, robustness and ruggedness studies. In addition, selectivity of the methods was studied by placebo blank and synthetic mixture analyses. A synthetic mixture is prepared based on the excipients present in the formulations and any interferer is eliminated by suitable solvent extraction.
Finally, a validated method was applied for assay to determine the active ingredient in dosage forms and the results obtained by the developed method were statistically compared with those of the reference method which is a pharmacopoeial method or a literature method by applying the Student’s $t$-test for accuracy and variance ratio $F$-test for precision. Lastly, accuracy of the developed methods was evaluated by performing recovery studies via standard-addition method.
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