1.0 Introduction:

Chemical degradation of drugs may result in altered therapeutic efficacy and even toxic effects. Pharmaceutical products are especially sensitive to variety of environmental factors such as temperature, humidity, light and oxidation. An extensive study on selected drug candidates was performed, where the study is still unknown. Pharmaceutical dosage forms of many drugs found to undergo facile degradation under different environmental conditions to yield different products which have toxic or adverse effects. It is clearly evident that, the analytical methods should be capable of indentifying and quantifying those products. i.e. the methods should be stability indicative, specific and selective to perform the study. Stress studies should be performed on drug candidates to assure the inherent stability of the drug and which inturn extended and extrapolated towards the regular stability of the drug product. The degradation pathways for all the drug candidates, which should really ease the pharmaceutical development of drug products containing respective drug substances and also provides strict storage conditions necessary for the maintenance of integrity including product activity.

1.1 The diseases, drugs and their pharmacological activity

Modern lifestyle has brought several comforts at the same time new diseases to the mankind. The living styles, food habits and alteration of local, regional and global ecosystems made human beings to prone to several kinds of diseases, diabetes, cancer and cardiac disorders which were once very rare and now effecting most of the people worldwide. Human beings generally face several challenges or difficult situations in life. Disease such as Cancer threatening the world. Rapid changes in life style put stress and pressure on human beings who in turn cause several disorders. The information on diseases cancer, Hypertension is given below linking the how the drugs under investigation are used in the treatment of these diseases.

1.1.1 Cancer, Hypertension diseases Treatment and Medication

Human beings generally face several challenges or difficult situations in life. The theoretical information on diseases cancer, Hypertension is given below how the drugs under investigation are used in the treatment of these diseases. Cancer is a disease of cells of organs and tissues of the body. Cells are constantly becoming old and dying,
and new cells are produced to replace them. Normally cells divide in an orderly and controlled manner. If for some reason the process gets out of control, the cells carry on dividing developing into a lump which is called tumor.

1.2 Lung cancer, Symptoms Treatment and medication

Lung cancer is the uncontrolled growth of abnormal cells that start off in one or both lungs, usually in the cells that line the air passages. The abnormal cells do not develop into healthy lung tissue, they divide rapidly and form tumors. As tumors become larger and more numerous, they undermine the lung’s ability to provide the bloodstream with oxygen. Tumors that remain in one place and do not appear to spread are known as “benign tumors”. The main types of lung cancer are small cell lung carcinoma (SCLC), also called oat cell cancer, and non-small cell lung carcinoma (NSCLC). Nearly 40% of lung cancers are adenocarcinoma [1]. A man’s risk of developing lung cancer is related to his age, genetics, Race, diet, lifestyle, medications, and other factors [2].

The symptoms of lung cancer are due to direct effects of the primary tumor, to effects of metastatic tumors in other parts of the body, or to disturbances of hormones, blood, or other systems caused by the cancer. Early lung cancer usually causes no symptoms. Symptoms of primary lung cancers include cough, coughing up blood, chest pain, and shortness of breath. Chest pain is a symptom in about one-fourth of people with lung cancer [3,4]. Treatment of lung cancer refers to watchful therapies, such as surgery, radiation therapy, chemotherapy, Targeted therapy and palliative care, [5] alone or in combination. There are a number of different treatment options for lung cancer. Standard treatment options include surgical resection, chemotherapy, and radiation therapy.

Medicines are used when cancer has spread from the lungs. They may also be given to certain men undergoing radiation therapy or surgery to prevent return of their cancer. Some of the medications used for the lung cancer are given in table.1.2.T1.
1.2 T1 Active pharmaceutical ingredients used for treatment of lung cancer

<table>
<thead>
<tr>
<th>Name</th>
<th>Mode of action</th>
<th>Pharmaceutical ingredient (API) examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targette Drug Therapies</td>
<td>Epidermal growth factor receptor (EGFR) Tyrosine kinase inhibitors (TKI's).</td>
<td>Erlotinib Hydrochloride</td>
</tr>
<tr>
<td></td>
<td>Monoclonal Antibody against EGFR</td>
<td>Cetuximab</td>
</tr>
<tr>
<td></td>
<td>Inhibitors of vascular endothelial growth factor (VEGF)</td>
<td>Bevacizumab</td>
</tr>
</tbody>
</table>

1.3 Chronic myelogenous leukemia, Symptoms, Treatment and medication

Chronic myelogenous leukemia (also called CML or chronic granulocytic leukemia) is a slowly progressing blood and bone marrow disease. People with acute leukemia have an increased number of immature white blood cells. Early symptoms of chronic myelogenous leukemia include chills, sweating, fever without infection, and fatigue. The increased numbers of white blood cells spill out of the bone marrow and start circulating in the blood stream and eventually become trapped in the spleen, causing it to enlarge [6,7]. Targeted drugs that block the action of tyrosine kinase include Imatinib (Gleevec), Dasatinib (Sprycel) and Nilotinib (Tasigna). Chemotherapy drugs are typically combined with other treatments for chronic myelogenous leukemia.

1.4 Hypertension Symptoms Treatment and medication

Hypertension, or high blood pressure, is one of the most common medical problems in the developed world. Unfortunately, hypertension often goes undiagnosed and when it is diagnosed it is often inadequately treated, despite the fact that it is usually fairly easy to treat. The aldosterone receptor antagonists (ARAs) spironolactone (Aldactone) and eplerenone (Inspra) have become part of standard medical therapy for heart failure, having shown clinical efficacy. The benefits include a lower rate of death. Many newer antihypertensive drugs have a slightly different chemical structure from older
drugs but produce nearly identical effects in the body. Several classes of antihypertensive drugs, diuretics, anti-adrenergics, direct-acting vasodilators, calcium-channel blockers, angiotensin-converting–enzyme (ACE) inhibitors, and angiotensin-receptor blockers (ARBs).

**1.4.1 Quality, safety and efficacy of drugs**

Drugs must be safe, effective, and of acceptable quality, in order to produce the desired action. The use of ineffective and poor-quality drugs will not only endanger therapeutic treatment but also erode public confidence in any country's health mission. The development of drugs is generally taken for granted along with their availability, feasibility, quality, safety, and efficacy. The role of technology in assuring the public that the drugs are of high quality is inevitable. Here the quality commands special attention because the medicinal products are effecting the life of human beings directly. Drugs are manufactured by appropriate chemical reactions of natural/mainly synthetic raw materials under controlled conditions. Presence of different kinds of impurities affects the quality of the drugs.

The impurities in drugs often possess unwanted pharmacological–toxicological effects by which any benefit from their administration may be outweighed [8-15]. The importance of carefully controlling the effects of even minor changes in the technology on the impurity profile and its consequence on drug safety can be characterized by an example related to the purity of certain industrial batches of Imatinib Mesylate. Imatinib Mesylate is a synthetically produced anticancer and used over the course of a decade as a treatment for certain cancers. The pharmacokinetics of Imatinib Mesylate was studied thoroughly [16-19]. Main epidemiological studies on the outbreak concluded those genotoxic impurities traced to contaminants of Imatinib Mesylate generated during an untested production process.
1.4.2 Quality control of drug substances

Until the beginning of the twentieth century drug substances and products were produced and sold having no control. Quality of the drugs was generally poor. Many of the drug products were patent medicines of dubious value. Some are harmful to health and addictive. In 1937, ethylene glycol was used as a vehicle for an elixir of sulfanilamide, which caused more than 100 deaths [28]. Another example was Thalidomide, a sedative and sleep inducing drug introduced in the early 1960s. It caused serious malformation in newborn babies of women who consumed it during early phases of pregnancy. Later it was realized the S-(–)-enantiomer of thalidomide possess teratogenic action and has no importance for the desired sedative or sleeping inducing property [29].

As a consequence of these differences in activity, optical isomers displaying undesired effects have to be guaranteed not to exceed specific limit by the manufacturers. It has caused great concern for the pharmaceutical industry as well as various regulatory agencies. Thereupon the food and drug cosmetic act (FDC) was revised requiring advance proof of safety and various other controls for new drugs. As an example for the necessity of the change in the attitudes of the drug manufacturers involves the case of one of the most classical drugs, aspirin. Its quality was characterized by titrimetric assay (saponification by sodium hydroxide and back titration of consumed base) using US, British as well as Indian pharmacopoeias [30-32]. A color test for free salicylic acid as the main impurity /decomposition product was included. Later, when HPLC became widespread in pharmaceutical analysis, it was found that in addition to salicylic acid, bulk drugs as well as formulations of aspirin [33,34] from various manufacturers contain three more impurities as shown in 1.4.2.F1. It was supposed that these impurities are capable of reacting with protein amino functions that are responsible for allergic reactions of aspirin [35]. It was apparent that all of the above impurities consume sodium hydroxide in titration method and their presence could not be detected by conventional techniques.

The analysis of impurities in drug substances, from the initial screening through the use of validated methods in routine quality control and quality assurance is becoming an increasingly challenging task along the pharmaceutical value chain.
1.4.2.F1 Chemical structures of aspirin and its impurities detected by HPLC

Impurities 1, 2 and 3 were not described in any of the pharmacopoeia but detected by HPLC in 1970s.

1.5 Origin of impurities

Impurity profiling describes a group of analytical activities aiming at the detection, identification, structure elucidation and quantitative determination of organic, inorganic and genotoxic impurities in drugs. Impurities in drugs are generally originated from various sources and phases of synthetic processes and preparation of pharmaceutical dosage forms. Since, there are several possibilities of synthesizing the drugs, it is possible that the same product of different sources may give rise to different impurities. Impurities could originate from any of the following steps during the drug synthesis.

1.5.1 Products of incomplete reactions during synthesis

It is quite possible that partial reactions could lead to the formation of impurities in the synthesis of antihypertensive drug Lisinopril. The functional group –CO-Phe is introduced into the molecule and then transformed to –CH₂-Phe by catalytic hydrogenation. Unreduced or partially reduced oxo group leads to oxo and hydroxy derivatives of lisinopril as impurities [36].
1.5.1.F1 Catalytic hydrogenation during Lisinopril and its impurities detected by HPLC

1.5.2 Last stage intermediate of synthesis

Impurities falling in this category are often called as potential or probable impurities. For example the last step in the synthesis of imatinib mesylate is the condensation of I with II which could be the probable impurities in the bulk drug material [37].

1.5.2.F2 Last stage intermediate of Imatinib Mesylate
1.5.3 Products of over reactions
In many cases the final reaction in the drug substance moiety formation is not selective and the reagent attacks the last intermediate in addition to the desired site. This can take place not only in the final step but also in the previous steps. The oxidation step in the synthesis of Rabeprazole is a simple example of this phenomenon [38].

1.5.3.1 Products of over reactions

![Diagram showing the synthesis of Rabeprazole and the formation of impurities](image)

1.5.4 Impurities originating from starting materials
Impurities present in the starting materials can also be sources for impurities of many drug substances. In such cases the impurities undergo same reactions as the main component leading to impurities. As an example, the appearance of the irbesartan [39].

1.5.5 Impurities originating from solvents
Pharmaceutical manufacturers must ensure that residual solvents and related contaminants in the drug products are below the stipulated as safe by regulation. In some case either the solvents or their impurities could be transferred during the synthesis leading to a host of impurities in the drug materials. As an example, during the synthesis of Imatinib mesylate, methane sulphonic acid reacts with solvents and give rise to two impurities [40].
1.5.6 Reagents, Ligands and Catalysts

The use of catalysts may lead to the formation of impurities. Potassium t-butoxide is used as a catalyst in the synthesis of Fluoxetine during which the catalyst reacts with one of the intermediate chlorobenzotrifluoride t-butyl ether as an impurity.

1.5.7 Products of side reactions

In majority of the cases the side reactions are unavoidable besides the main reaction in organic synthesis. In the synthesis of quetiapine, the formation of dimeric impurity is a frequent phenomenon.
1.5.8 Degradation Products

Drugs are exposed to stressful conditions such as thermal, humidity and light in the environment during storage. Under these conditions some of the drugs undergo chemical degradation and gives degradation and gives degradation products as impurities. Resperidone on exposure to light or heat undergo degradation and one degradation product was formed. These degradation products were considered to be the impurities of finished products [41-42].

1.5.9 Enantiomeric impurities

Chiral pharmaceutical compounds are bulk materials, which are chiral in nature. Most natural organic products, the essential products of life, are asymmetric and such that they are not super impossible on their images, means they are chiral in nature”. Chirality is a major concern in the modern pharmaceutical industry. In the case of a chiral drug administered as a enantiomer, the antipode is considered to be an impurity [43-44]. Some examples of pharmaceuticals where one isomer has the desired effect and the other has properties include, thalidomide, where the R-enantiomer is a sedative and the S-enantiomer is teratogenic, ethambutol where the S,S-enantiomer is tuberculostatic and the R,R-enantiomer causes blindness. Table 1.5.9.T1 gives the list of some of the chiral drugs whose antipodes possess undesired effects.
1.5.9.1 Chiral Antipodes having undesried side reactions

<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical Structure</th>
<th>Configuration</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalidomide</td>
<td><img src="image" alt="Thalidomide" /></td>
<td>R, S</td>
<td>Sedative Teratogenic</td>
</tr>
<tr>
<td>Ethambutol</td>
<td><img src="image" alt="Ethambutol" /></td>
<td>S,S, R,R</td>
<td>Tuberculostatic Blindness</td>
</tr>
<tr>
<td>Pencillamine</td>
<td><img src="image" alt="Pencillamine" /></td>
<td>S, R</td>
<td>Antiarthritic Mutagen</td>
</tr>
<tr>
<td>Propranolol</td>
<td><img src="image" alt="Propranolol" /></td>
<td>S, R</td>
<td>Alpha Blocker Contraceptive</td>
</tr>
<tr>
<td>Barbiturates</td>
<td><img src="image" alt="Barbiturates" /></td>
<td>S, R</td>
<td>Depressant Convulsant</td>
</tr>
<tr>
<td>Labetalol</td>
<td><img src="image" alt="Labetalol" /></td>
<td>S,R-isomer, R,R-isomer</td>
<td>Anti coagulant Toxic</td>
</tr>
</tbody>
</table>

1.5.10 Residual solvents

Solvents are practically used in all phases of activities in the pharmaceutical industry. Residues of solvents are therefore usually present at trace levels in Bulk drugs and their subsequent formulations. Based on safety considerations, the solvents are placed in one of the classes given below [45].
1.5.10.1 Class1 residual solvents

The solvents are known as human carcinogens and to be avoided e.g. benzene (2ppm), carbontetrachloride (4ppm), 1,2-dichloroethane (5ppm) 1,1-dichloroethene(8ppm) and1,1,1-thichlorethane(1,500ppm). These suspected human carcinogens and environmental hazards should not be used in the manufacture of drug substances, excipient or drug products. If their use unavoidable, the levels should comply with the specifications prescribed by regulatory authorities.

1.5.10.2 Class2 residual solvents

The solvents are to be used in limited quantities in all processes of manufacturing. These are non-genotoxic animal carcinogens and should be Limited due to their inherent toxicity e.g.acetonitrile (410ppm), cyclohexane (3880ppm), methanol (3000ppm) etc. The values given in parenthesis of class 1 and class 2 solvents are threshold values.

1.5.10.3 Class3 residual solvents

These solvents are generally low toxic potential to man. No health based exposure limit is needed since the per day exposures (PDEs) can generally be limited by good manufacturing practices (GMP)or other quality-based requirements. E.g.acetic acid, ethyl acetate, ethanol, Tetrahydrofuran etc.

1.5.10.4 Inorganic impurities

There are various possible sources for inorganic impurities in drugs. The starting materials, catalysts, reagents and solvents could be the sources of inorganic salts.

1.5.11 Genotoxic impurities

Genotoxic impurities are gaining more attention in the pharmaceutical industry. These substances add risk without any benefit to pharmaceuticals. Genotoxic substances are those which impact genetic material by means of mutations. These changes to the genetic material, which can be caused by exposure to very low levels of a genotoxin, can lead to cancer [46].

Because of this, it is important to identify genotoxic substances followed by monitoring and control at very low levels (ppm or ppb) to ensure safety to the public. Genotoxic impurities in relation to pharmaceuticals can come from many places including starting materials, reagents, intermediates, solvents, reaganets or unwanted side reactions from the API synthetic process that get carried over into the final product. In addition, the
API itself can decompose to form genotoxic impurities or they can form in the drug substances by reaction between materials and the API. This highly noticed incident helped bring more attention to the issue of genotoxic impurities in pharmaceuticals. A major challenge with genotoxic impurities in pharmaceuticals is the balance between appropriate control with minimal impact to the time and costs associated with developing life-improving drugs [47-48].

1.5.12.F1 Genotoxic Impurity Decision Tree in Pharmaceuticals

1.6 Impurities in excipients

The most frequently used excipients in pharmaceutical formulations are carbohydrates e.g. lactose, starch, cellulose derivatives, polymeric materials such as polyethylene glycol, povidone, various oils of plant origin, stearic acid, its magnesium salt, inorganic materials such as calcium phosphate and various forms of salicylic acid etc. In majority of the cases the quantity of these materials are higher than that of the active ingredient. Traces of metals in the excipients can catalyze the degradation of the active
ingredients. For example, peroxide impurities in polyethylene glycols may cause the degradation of oxidizable drugs [49].

1.7 Impurity profile and control in Pharmaceuticals and significance

Impurity profiling describes a group of analytical activities aiming at the detection, identification, structure elucidation and quantitative determination of organic and inorganic impurities in drugs. Impurity profiles of drug substances include identification of the main impurities of intermediates, degradation products. Enantiomeric impurities and Quantitative determination of residual solvents and inorganic impurities [50]. It is quite essential to ensure that no new impurities appear in the course of scaling up procedure and also that the quantity of Impurities appear in the course of scaling up procedure and the synthetic Research phase, remain below the specification limits.

The identifications, structure elucidation and quantitative determination of Impurities and degradation products are of prime importance in the course of all the phases of research, development and production of drug formulations. Stability-indicating analytical methods are to be used in the course of development of drug formulations. These studies indicate which of the Impurities in the bulk drug are of the degradation type. During the stability studies the content of these degradation products increases while the synthesis related impurities are likely to remain constant. The comparison of the impurity profiles of several batches from the same manufacturer provides a good indication for the consistency of manufacturing process while the comparison of samples originating from different manufacturers can give a clear picture about the differences between their purity and the levels of the manufacturing procedures.
1.8 Strategies in impurity profiling

It is difficult to generalize the strategy regarding impurity profiling in different laboratories (1.8.F1) shows the systematic use of analytical techniques for impurity profiling of drugs. Although the same techniques are used in all the laboratories, the manner of their use in individual laboratories could be quite different. The instrumentation used in the impurity profiling is very rapidly increasing. This can be characterized by the complexity of scheme for the impurity profiling published in the literature [51,52]. The introduction of hyphenated techniques like LC-MS-MS, LC-NMR etc. In the late 1990s created an entirely new situation in pharmaceutical research and analysis and also in the field of impurity profiling. The hyphenation of chromatographic methods with spectroscopic techniques really enables even minor impurities to detect, identify and characterize within a short time with certainty. The understanding, identification, quantification and control of impurities in drug substances are essential as new molecular entities are evaluated in pharmaceutical development. As chemical processes used to produce drug substances mature from the early phases of development through registration, a concomitant maturing of process-related impurity understanding and control is required. Methodology aspects for impurity investigations are important along with an emphasis on understanding the origin and fate of impurities to guide decisions on process controls and specifications.

Special considerations are necessary for stereochemical impurity investigations. Considerations for selective analytical methodology and determination of the process capability for removing the impurity are necessary. Routine analytical testing for a toxic impurity was not required where a high impurity rejection efficiency of the synthetic process. Quality assessment of starting materials from multiple sources and the impact of starting material impurities on the impurity profile of the drug substance needs to be established 1.8.F1.
1.8.F1 A General scheme for detection, identification, structure elucidation and determination of related organic impurities in drugs.

Detection by TLC,HPLC,(GC,CE,CEC,SFC)

Retention matching with authentic samples of potential impurities in 2-3 chromatographic system

Identified ?

No

Unidentified impurities

UV(HPLC,CE,CEC/DAD)
UV,IR(TLC reflection spectra)

Information sufficient

No

NMR,MS without Chromatography

Yes

Suggested Structure

Synthetic of the Impurity

Retention matching

Identified impurities

Method development for quantitative determination

Yes

HPLC(CE,CEC,SFC)MS

GC(IR)MS

MS(IR)

Information sufficient

No

Semipreparative TLC

Small sample from HPLC

Sample from preparative HPLC or TLC

HPLC/NMR(WS)

Integrated information
UV,IR,MS,NMR
Chromatographic data

NMR
To ensure the quality, safety and efficacy of pharmaceutical products, most nations have enacted laws and regulations governing the production, manufacturing, import and sale of drugs. Many countries, the council of Europe and the World Health Organization (WHO) have established pharmacopoeias containing specifications for drugs, including purity, and the methods of analysis to verify the specifications.

1.9 Pharmacopoeial status and regulatory aspects

The USP and NF have become official upon adoption of the first food and drugs act. They formulated legal standards of quality, purity and strength of new drugs. The good manufacturing practices provide minimum quality standards for production of pharmaceuticals as well as their ingredients [53]. The international conference on the Harmonization of the Technical requirements for registration of pharmaceuticals for Human use (ICH) which took place in Yokohama, Japan in 1995, has released new guidelines on impurities in new drug products [54-55] have a number of advantages, both for the industry and the regulators.

1.9.1 ICH guidelines for identification and qualification of impurities

<table>
<thead>
<tr>
<th>Dose</th>
<th>Threshold for Identification(%)</th>
<th>Qualification(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 mg</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1-10 mg</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>1-100 mg</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>100-2g</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>&gt;2 g</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
A rationale for selecting impurity limits based on safety considerations has to be provided. The "Decision tree for safety studies" (Figure 1.9.F1) describes consideration for the qualification of impurities when thresholds are exceeded. This has some consequences for method development. Analytical procedures should be able to separate all the impurities from each other and the method should be optimized to separate and quantify them in the dosage forms. Such methods are to be validated demonstrating the accuracy, precision, specificity, limit of detection, quantitation, linearity, range and interferences.

The validation of analytical procedures i.e., the proof of its suitability for the intended purpose, is an important part of the registration application for a new drug [56,57]. The ICH has harmonized the requirements in two guidelines [58,59]. The first one summarizes and defines the validation characteristics needed for various types of test procedures, the second one extends the previous text include the experimental data required and some statistical interpretation. These guidelines serve as a basis worldwide both for regulatory authorities and industry to bring the importance of a proper validation to the attention of all those involved in the process submission of drug master files (DMF).

The analytical research and development units in the pharmaceutical industry are responsible for preparation and validation of test methods. In the last few years there has been an increase in importance for characterization and quantitative determination of impurities in bulk drug materials and pharmaceutical products.

The appearance and spreading of a broad and sophisticated array of spectroscopic, chromatographic and electrophoretic techniques as well as their hyphenated combinations allow determination of structures and concentration of related organic impurities (degradation products), residual solvents, inorganic impurities down to ppm level within a short time. This would not have been possible to imagine 10-20 years ago. Separation methods are developed to detect and measure virtually any impurity, which is present at levels of detection. A major challenge for development of more potent drugs is development of new methods for their analysis. This situation has
become especially critical for the analysis of impurities in the drugs, since the toxicity of these may be such that the impurities must be controlled to extraordinary low levels.

Another important aspect to the development of modern pharmaceuticals has been the ability of researchers to develop methods to follow the metabolic paths of drugs in the human body. It is certain that a full understanding of the action of a drug substance cannot be obtained without determination of the concentration of the active form of the drug in the body as time progress.

1.9. F1 Decision tree for qualification of impurities in drugs and pharmaceuticals.
1.10 Evolving concepts for use of analytical methodology

From the analytical point of view, understanding the identification, quantification and control of impurities in drug substances are essential as new molecular entities are evaluated in pharmaceutical development. As chemical processes used to produce drug substances mature from the early phases of development through a process related impurity understanding and control. Chromatographic purity analyses are a crucial part of drug development and quality control in pharmaceutical laboratories. Many of the anti-cancer drugs are themselves cytotoxic and their synthetic and organic impurities are equally so or sometimes these impurities are genotoxic and also degradants. The old concept to detecting and estimating impurity levels not exceeding 0.1% cannot be applied, which might have to be measured at levels of 0.01% or even 0.001% [20-23].

To reach such low analytical limits, advanced techniques and methodologies have to be developed. Sophisticated and highly sensitive methods have been developed to ensure the quality of the drugs. The quality, efficacy and safety of the drug is generally assured by monitoring and controlling the impurities effectively. Thus the analytical activities concerning impurities in drugs are among the most important aspect in modern pharmaceutical analysis. This has become quite evident by the certain publications on this topic [24-27].

1.10.1 Interdisciplinary use of Analytical Methodology

The development of a drug dosing regimen cannot be undertaken without knowing how long the compound remains in circulation at therapeutic levels, and this information can only be gleaned from studies of drug concentration levels in body fluids. This situation has become even more important when one considers that drug substances are becoming progressively more potent, making it essential that workers fully understand the kinetics of drug action. The use of an analytical method has permitted the development of drugs for which the information could not have been obtained by any other techniques. The measurement of Enantiomeric purity at ever-decreasing analytical levels will be a challenge for the future. For the last few years,
the use of HPLC has gone from being a laboratory curiosity to the current state where HPLC is the workhorse of all analytical facilities.

Recently capillary electrophoresis (CE) has begun to be used and reported that it can reach even higher levels of sensitivity. It is anticipated that new methods of separation and detection will continue to be developed by ingenious scientists and that these advances will push our ability to detect minute levels of impurities even further. Methods development optimization is typically continued until the objectives outlined in the development plan are met. Once the method is determined to be optimum, the method is evaluated to see if it will meet validation requirements. In this exercise, the method is challenged in some of the following areas. Whether the method is selective and Meeting the target LOQ. Placebo, stressed placebo issues. Known impurities Degradants are able to detect. Peak purity in diode array and/or LC-MS and Linearity.

1.11 Requirements of an Analytical Methodology

At various stages of the drug development process, release and stability testing is required to guarantee the quality and safe use of a new-marketed medicinal product. The quality parameters and stability requirements imposed to the drug substance and drug product must be carefully followed-up by means of appropriate and validated analytical methods. The filed methods must be simple, robust, and reliable—that is, easy to use and perform without deviations when appropriately applied in a qualified laboratory. Although many quality attributes of the drug substances need to be controlled by a variety of analytical methods, this study will focus on the development of an UPLC methods for the assay and related impurities and identification of degradants by LC/MS.

The developed methods should be applicable for both release and stability purposes. Special attention must be given to the robustness of the analytical methods, especially for methods that will ultimately be transferred from a development laboratory to quality control sites and stability laboratories. Robust analytical method is a swift check on the performance of a method at various application laboratories. In this evaluation test, prior to formal validation and method transfer, some qualitative aspects of the method— for example, a visual check of the baseline, selectivity of the methods toward the
specified impurities and degradants, estimation of the limit of quantification, and so on are examined in the control laboratory. This preliminary evaluation can be used to improve the quality of the method through understanding possible method performance deficiencies and enable sufficient time for the concerns to be addressed prior to method validation and transfer.

1.12 Importance of Drug substance Stability

Chemical compounds can decompose prior to chromatographic investigations, for example, during the preparation of the sample solutions, extraction, cleanup, phase transfer or storage of prepared vials (in refrigerators or in an automatic sampler). Stability testing is important to evaluate an analytical method’s ability to measure drug products in the presence of its degradation products.

The purpose of stability testing in pharmaceutical industries is to provide evidence on how the quality of a drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommended storage conditions, retest periods, and shelf lives to be established. The goal of stability program depends on the stage of development of the drug product.

At this stage the effect of pH, moisture, air (oxygen), heat and light on the stability of the drug substance is also studied. The accelerated stressing of the drug substance and drug product provides information to the intrinsic stability of the molecule/formulation and may establish likely degradation pathways. On the analytical side, the analytical research group supports the pre-formulation stability program, which is ultimately responsible for developing and validating the stability-indicating assay/related substances. The marketed product stability program fulfills the commitment part of the NDA and also ensures that the marketed drug products are stable (potent) until the expiry date stamped on the product label.

1.13 Contribution of Analytical methods during drug development

The contributions of analytical testing during the drug development process in each stage are listed in figure 1.8.F1. Suitable and capable test methods are required to ensure accurate and reliable data at each stage of the process. Regulations and quality
standards are dynamic. They are updated every few years. Implementation guidelines, as developed by regulatory task and industry task forces, are published more frequently since state-of-the art today may not be considered as such in the future.

1.14 Analytical Methods Development

After attaining to the plan, resources, and the objectives of the analytical method are optimised, the laboratory portion of the method development process begins. In brief, at the initial stage of development of assay/purity method, typical chromatographic conditions are evaluated in order to provide resolution of the related substances from the active drug substance or individual would also need to be chromatographed to determine if they interfere. Once basic resolution criteria are met (able to separate the related substances from the active), other laboratory method development activities are pursued including evaluation of sample preparation. If the method does not meet run time criteria or robustness requirements, it may require further optimization. Recently, many laboratories have found success using computerized method optimization software.

Several commercial packages are available that combine classical chromatographic theory with statistical design to predict optimum separation conditions with a minimum number of experiments. Methods development optimization is typically continued until the objectives outlined in the development plan are met. Once the method is determined to be optimum, the method is evaluated to see if it will meet validation requirements. In this exercise, the method is challenged in some of the areas like target LOQ, selective method, Peak purity diode array and/or LC-MS and Linearity. Successful completion of pre-validation assessment suggests that the method is capable of entering the much more rigorous validation stage.

1.14.1 System Suitability

An analytical test method was always expected to perform in an acceptable manner each time it is used. System suitability testing ensures that the total system is functioning to the expectations at the given time. System suitability testing, coupled with previous instrument qualification, periodic calibration, and method validation, provides assurance that the test method will provide accurate and precise data for its intended use. Properly chosen system suitability criteria will fail just prior to the point where the system
will begin to produce less acceptable data; however, criteria should not be so restrictive that acceptable data cannot routinely be used. It is the challenge of the method development scientist to develop realistic and meaningful system suitability criteria.

As implied above, only meaningful system suitability criteria and those required by in-house or regulatory policy should be evaluated. During the method development process and robustness evaluation, marginal performance of the system can be observed. System suitability should be monitored over time to verify that the criteria remain realistic and achievable while continuing to provide assurance of the suitable performance of the method.

1.14.1.T1 System suitability tests associated validation parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>System suitibaility test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precesion</td>
<td>RSD of Five six injections of standard</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Retention factorNumber of theoretical plates Tailing factor Resolution (injection of a resolution mixture)</td>
</tr>
<tr>
<td>LOD/LOQ</td>
<td>Injection of a dilution of the standard to verify LOD/LOQ</td>
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</table>
1.15 Analytical Method Validation

In its basic form, analytical method validation is matter of establishing documented evidence that provides a high degree of assurance that the specified method will consistently provide accurate test results that evaluate a product against its defined specification and quality attributes. The process is broken down in phases because of the length and time of complexity. Properly executed, the validation process can be as significant an effort as method development. The activities must be properly planned to ensure an efficient and successful validation. The validation stage of method development is a confirmation process, there should be few “surprises” in validation results, because pre validation evaluation data should suggest that the method would validate successfully. While the requirements of validation have been clearly documented by regulatory authorities, the approach to validation is varied and open to interpretation. The approach below will focus on International Conference on
Harmonization (ICH) guidelines. A generalized flowchart of the validation process is detailed in 1.15.F1.

1.15.F1 Method Validation-Flow Chart
1.15.1 Analytical Method Validation—Definitions

It is important to define the terms used in regulatory guidance when discussing method validation. The definitions listed below are quoted directly from the ICH guideline.

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.” “Accuracy studies measure the effects of the sample matrix on the values obtained and are often carried out in the context of recovery studies”.

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

Repeatability: “Repeatability expresses the precision under the same operating conditions over a short interval of time.”

Intermediate Precision: “Intermediate precision expresses within laboratory variations: different days, different analysts, different equipment, etc.”

Reproducibility: “Reproducibility expresses the precision between laboratories.”

Specificity: “Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.”

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.”

Quantification Limit: “The quantification 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”.

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

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

Ruggedness: The USP defines ruggedness as “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 labs, different analyst, different lots of reagents. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

Sensitivity: The sensitivity of an analytical method is equal to the slope of the calibration line in a linear system.