CHAPTER 1

Introduction to Active pharmaceuticals ingredients, impurity profiling and analytical methods/techniques applied

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1.1 Active pharmaceutical ingredients, its impurity profiling and overall analytical role:

Active Pharmaceutical Ingredient (API) is a substance intended to be used in the manufacture of a drug (medicinal) product and is responsible for eliciting the desired pharmacological activity. Such substances are generally called drug substances and used to formulate the drug product which are consumed by the patients. These drug products furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the body. Thus, pharmaceutical sector has two demarcated sections, one is manufacturing of API’s and other is formulating them in the tablets, capsules, injectables, syrup, patches, etc. International Conference of Harmonization has adopted stringent rules to maintain quality of the API used in pharmaceutical product. Quality of the drug is nothing but compliance of the substance (API) with respect to set of quality parameters such as chemical purity, chemical assay, impurity profile, content of known and unknown, and genotoxic impurities, inorganic contents, residual solvents, physical attributes such as polymorph, particle size, bulk density and etc. All these set of analytical examination is formatted together and formatted to release the material to end users is known as Certificate of Analysis (COA). Establishment of analytical chemist is multifold and critical during the period of product or process development. The role of analytical chemist in the pharmaceutical industry plays an extremely important role in developing analytical methods that ensure the safety, efficacy, purity, stability and overall quality of the API and formulated drug products. Method validation of analytical methods is another milestone,
which is critical to ensure that impurity levels are accurately and consistently reported throughout drug development.

Most of the active pharmaceutical ingredients (API’s) are produced by organic chemical synthesis. Various components, including residual solvents, trace amounts of inorganic and organic components can be generated during such process. Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (API’s), or develop during formulation, or upon aging of both API and formulated API’s to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Hence impurity profiling is a very important document and hence gaining critical attention from regulatory authorities. The impurity profile can be defined as “A description of the identified and unidentified impurities present in a new drug substance”.

The different pharmacopoeias, such as the European pharmacopoeia (EP), British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to the tolerance levels of impurities present in the APIs or formulations. The International Conference on Harmonization (ICH) is a triplicate body of United States, European Union and Japan. The ICH Steering Committee includes observers from WHO (World Health Organization), Health Canada and the European Free Trade Association. ICH has published guidelines for impurities present in new drug substances, products and residual solvents. According to ICH guidelines for impurities in new drug products, the drug with a maximum dose of less than 1g/day the identification threshold for unknown impurity is 0.10%. Between 1- 2 g/day the identification threshold is 0.1-0.05% and above 2 g/day it is 0.05%.
According to ICH guidelines, impurities associated with APIs are classified into the following categories:

- **Organic Impurities**
- **Inorganic Impurities**
- **Residual solvents**

Of the above three classified groups, the number of possible inorganic impurities and residual solvents are limited; these are easily identified and their physiological effects and toxicity are well known. For this reason the limits set by the pharmacopoeias and the ICH Guidelines can guarantee that the harmful effects of these impurities do not contribute to the toxicity or side effects of the drug substance. However, such effects can not be outlined for organic impurities, as these compounds are prepared by multistep synthesis. Number of impurities and their formation are almost unlimited and highly dependent on the route and reaction conditions of the synthesis. It also depends upon several other factors such as the purity of the starting material, method of isolation, purification, condition of storage, etc. In addition to this, their toxicity is unknown or not easily predictable hence organic impurity studies in API’s play a vital role in drug development process.

Organic impurities may arise during the manufacturing process and/or storage of the drug substance. They may be identified or unidentified, volatile or non-volatile, and includes starting materials, by-products, degradation products, reagents, ligands, catalysts, and enantiomeric impurities etc.
To meet the strict guidelines applied by various authorities, it has become mandatory to monitor all impurities to the lowest possible level and hence eventually need is generated to carry out detailed impurity profile for active pharmaceutical ingredients. Impurity profiling is of utmost importance in all phases of synthetic drug research and development from the gram scale preparation to commercial level production. The latter aspect should be emphasized more, since even minor changes in the production technology, source of starting materials, conditions of purification and storage can greatly influence the impurity profile.

1.2 Analytical methods/techniques used for impurity profiling study:

Identification, characterization and quantitative determination of impurities (and degradation products) in APIs and pharmaceutical formulations is one of the most important activities in modern pharmaceutical analysis. The reason for the increased importance of this area is that unidentified, potentially toxic impurities are health hazardous and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods.

The separation, identification and determination of impurities to lowest possible level in drug substance are done by various techniques. The separation techniques include various chromatographic techniques. These techniques are based on the separation of a mixture of species in a sample due to differential migration. Now-a-days, impurity profiling studies in drug substances are carried out by different spectroscopic (Mass spectrometry and Nuclear magnetic resonance spectroscopy) and chromatographic techniques (TLC, HPLC, GC, LC-MS and GC-MS). The application of these
techniques is dependant on the nature of impurities and API’s. Quantification of impurities in drug substances is a need of pharmaceutical industry.

In all chromatographic separations the sample is dissolved in mobile phase, which may be a gas, liquid, or a supercritical fluid. This mobile phase is then forced through an immiscible stationary phase, which is fixed in a column or on a solid surface. The two phases are chosen so that the components of the sample distribute themselves between the mobile phase and stationary phases to varying degree. The components which are strongly held by the stationary phase moves slowly whereas the components which are weakly held by the stationary phase moves faster and migrates to detector. Due to these differences in migration rates, sample components separate into discrete bands or zones, which can be analyzed quantitatively and qualitatively.

The classification of chromatographic methods is based on the type of mobile phase, type of stationary phases and the type of equilibria involved in the transfer of components between phases. Based on the above mentioned criteria, there are three general categories of chromatography: gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SFC). In GC, LC, and SFC techniques gases, liquids, and supercritical fluids, respectively are the mobile phases.

In the present work, analytical chemistry research conducted to develop proper analytical methods for analyzing raw materials, intermediates, in-process checks, and impurity profiling while developing the process for the preparation of API’s followed their validation. The impurity profiling study of active pharmaceutical ingredient is carried out by GC and LC methods. Generally, the LC and GC techniques are mostly used for
impurity profiling studies in drug substances because of simplicity, easy availability, less expensive and readily adapted to quantitative analysis.

A brief discussion of the instrumentation and technique of high performance liquid chromatography (HPLC) and gas chromatography (GC) used in the present research work are discussed below.

1.2.1 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC)\textsuperscript{21-23} is a physical separation technique conducted in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is now one of the most powerful techniques in analytical chemistry and it has been most widely used for the quantitative analysis of pharmaceuticals, biomolecules, polymers, and other organic compounds. It has the ability to separate, identify, and quantitate the constituents that are present in any samples that can be dissolved in a liquid. The trace concentrations as low as parts per trillion [ppt] of impurities in sample may easily be identified and quantified.

In normal-phase chromatography, it is used a polar stationary phase and a less polar mobile phase. However, in reversed-phase chromatography, the stationary phase is non-polar or weakly polar and the mobile phase is more polar. The reversed-phase chromatography eliminates peak tailing because the stationary phase has few sites that can strongly adsorb a solute to cause tailing. Reversed-phase chromatography is also less
sensitive to polar impurities in the eluent, such as water. The reversed-phase chromatography uses columns that are 5-30 cm in length, with an inner diameter of 1-5 mm and the column is packed with small size particles that are from 3-10 mm. The small the particles higher is the efficiency but require a high pressure. Such particles form the stationary phase that consist of microporous particles of silica that are permeable to solvent and have a surface area of several hundred square meters per gram. There are two kinds of elution that can be done in reversed-phase chromatography named as isocratic and gradient elution.

In isocratic elution compounds are eluted using constant mobile phase composition. Isocratic analyses are particularly common in quality control applications since they use simpler HPLC equipment and premixed mobile phases. The disadvantages of isocratic analysis are limited peak capacity and problems with samples containing analytes of different polarities. Also, late eluting solutes (such as dimers) are particularly difficult to quantitate in isocratic analysis due to excessive band broadening with long retention time. Optimizing conditions for late eluting solutes, on the other hand, may provide an inadequate separation of early eluting solutes. Changing the composition of the mobile phase with time provides a solution to this problem. In a reverse-phase separation the initial mobile-phase composition is relatively polar. As the separation progresses, the mobile phase’s composition is made less polar. Such separations are called gradient elutions.

Gradient analysis is suited for complex samples and those containing analytes of wide polarities. Gradient chromatography is amenable for high-throughput screening applications and for impurity testing. The major advantages of gradient analysis yields
better separation for early peaks and sharper peaks for late eluters. The disadvantages of gradient analysis are the requirements for more complex instrumentation and greater skills in method development, and difficulties in method transfer.

1.2.1.1 The Basic Parts of a HPLC Instrumentation

HPLC instrumentation includes a pump, injector, column, detector and data system. A schematic diagram of a typical HPLC instrumentation is shown in Figure-1.1.

![FIGURE-1.1. Basic HPLC instrumentation](image)

The heart of the system is the column where separation occurs. Since the stationary phase is composed of micrometre size porous particles, a high pressure pump is required to move the mobile phase through the column. The chromatographic process begins by injecting the solute onto the top of the column. Separation of components occurs as the analytes and mobile phase are pumped through the column. Eventually, each component elutes from the column as a narrow band (or peak) on the recorder. Detection of the eluting
components is important, and this can be either selective or universal, depending upon the detector used. The response of the detector to each component is displayed on a chart recorder or computer screen and is known as a chromatogram. To collect, store and analyse the chromatographic data, computer, integrator, and other data processing equipment are frequently used. The details of HPLC instrumentation part are as discussed below;

1.2.1.1.1 Reservoir of mobile phase

The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with the special caps, teflon tubing and filters, to connect to the pump inlet and to the purge gas (He) which is used to remove dissolved air.

1.2.1.1.2 Pump (Solvent delivery system)

Pumps are used to deliver the mobile phase to the column. Different types of pumps are used to maintain flow rate and pressure of the mobile phase. The pump, its seals, and all connections in the chromatographic system must be constructed of materials that are chemically resistant to the mobile phase. Also a degasser is needed to remove dissolved air and other gases from the solvent. A degassing unit is required to remove dissolved gases from the mobile phase. A desirable feature of the delivery system is the capability of generating solvent gradient. A pump should be able to operate up to a pressure of 100 atm (1500 psi) though in some cases 400 atm (6000 psi) is desired. For most analytical columns, only moderate flow rates of 0.5-5 mL/min may be required. However, for microbore columns, low flow rates of only a few microlitres/min may be sufficient. Also, a pump should have a small hold up volume. The function of pump in HPLC is to pass mobile phase through the column at high pressure and controlled flow rate.
1.2.1.3 Injector

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. In more sophisticated LC systems, automatic sampling devices are incorporated where the sample is introduced with the help of autosamplers and microprocessors. In liquid chromatography, liquid samples may be injected directly and solid samples need only be dissolved in an appropriate solvent. The solvent need not be the mobile phase, but frequently it is judiciously chosen to avoid detector interference, column/component interference, loss in efficiency or all of these.

It is always best to remove particles from the sample by filtering over a 5 \( \mu m \) filter, or centrifuging, since continuous injections of particulate material will eventually cause blockages in injection devices or columns. These devices form an integral part of LC equipment having interchangeable loops with a choice of sample size from 5 to 500 \( \mu \)L. The availability of highly sensitive detectors frequently allows use of the small samples which yield the highest column performance.

1.2.1.4 Column of column configuration and column chemistry

An accurate choice of column configuration and column chemistry is very important for carrying out pharmaceutical analysis by HPLC. It is the heart of the HPLC instrument where actual separation occurs. It is the central component of HPLC. HPLC columns are available in various lengths and diameters. To withstand the high pressures involved, columns are constructed of heavy-wall; metal tubing or smooth-bore stainless-steel tubing which are inert to the chemical corrosion due to mobile phase. Typical HPLC columns are 5, 10, 15 and 25 cm in length and are filled with small diameter (3, 5 or 10 \( \mu m \))
particles. The internal diameter of the columns is usually 4.6 mm; this is considered the best compromise for sample capacity, mobile phase consumption, speed and resolution.

1.2.1.1.5 Detector

A detector is an important part of the HPLC instrument for detection and quantitation of solute. There are various types of detector available with HPLC instrumentation. Even if highly sensitive detectors have been developed for HPLC, still there is no universal detector which could be used for all kinds of samples and for all concentration ranges. The choice of a detector depends on the nature of the sample. There are two basic types of detectors such as Bulk-property detector and solute property detector. A bulk-property detector responds to an overall change in a physical property (refractive index, dielectric constant, density, electrical and thermal conductivity, vapour pressure etc.) of the mobile phase with and without solute. These types of detectors are somewhat less sensitive and require good temperature control. In contrast, solute-property detectors only respond to a physical property of the solute, such as absorbance, fluorescence, or diffusion current, which is not showed by the mobile phase. These types of detectors are highly sensitive with a detection signal for a few nanograms of sample. In this category absorbance, fluorescence, diffusion current and electrochemical detectors are considered. Generally, most commonly used detectors are a variable wavelength ultraviolet-visible spectrophotometer, fluorescence detector, refractive index detector, or an electrochemical detector. Mass spectrometry detectors are currently quite popular for identification of solutes.
1.2.1.6 Recorder

Since the detector signal is electronic, using modern data collection techniques can aid the signal analysis. In addition, some systems can store data in a retrievable form for highly sophisticated computer analysis at a later time. The main goal in using electronic data systems is to increase analysis accuracy and precision, while reducing operator attention. There are several types of data systems, each differing in terms of available features. In routine analysis, where no automation (in terms of data management or process control) is needed, a pre-programmed computing integrator may be sufficient. If higher control levels are desired, a more intelligent device is necessary, such as a data station or minicomputer. The advantages of intelligent processors in chromatographs are found in several areas. First, additional automation options become easier to implement. Secondly, complex data analysis becomes more feasible. These analysis options include such features as run parameter optimisation and deconvolution (i.e. resolution) of overlapping peaks. Finally, software safeguards can be designed to reduce accidental misuse of the system.

1.2.1.2 Stationary Phases in RP-HPLC

Selecting an appropriate stationary phase can also help to improve the efficiency of method development in HPLC analysis. The stationary phase can be a solid, a liquid, or a bonded phase i.e. stationary phase chemically bonded to a support that is used for the separation. The most common columns are packed with silica particles. The silica is the most widely used as the base material for bonded phases.\textsuperscript{24} The alkyl silanes materials are most widely used as bonded phases which are coated on a packing material consisting of 3-10 µm porous silica particles.\textsuperscript{25} Polymethylmethacrylate (PMMA),
poly-styrene-divinyl benzene (PS-DVB), methacrylate, hydroxyethylmethacrylate (HEMA), alumina, carbon, and other polymeric and inorganic materials are also used as base materials. The particles may be regular or irregular in shape. Spherical particles are more popular, as are the smaller particle sizes (usually about 3, 5, or 7 µm). The particle size plays a vital role on separation. Larger particles will generate less system pressure and smaller particles will generate more pressure. The smaller particles generally give higher separation efficiencies. It is very important that stationary phases exhibit good long-term stability when used with highly aggressive mobile phases.

The stationary phase may be partially soluble in the mobile phase, causing it to “bleed” from the column over time. To prevent this loss of stationary phase, it is covalently bound to the silica particles. The hydrocarbon ligands are coupled to the silica gel via silanol groups on the silica surface using organochlorosilane reagent of the general form Si(CH₃)₂RCl, where R is an alkyl or substituted alkyl group (Figure-1.2).

![Figure-1.2](image)

**FIGURE-1.2.** Substitution of silica with alkyl chains by reaction with organosilane reagent.

After the functional group has been bonded to the silica surface, there are residual unbonded silanol groups on the silica surface that have not reacted due to steric hindrance. In these instances the residual silanol groups can interact with basic and/or polar
compounds and result in tailing peaks, which make quantitation difficult. To prevent this unwanted interactions between the solutes and any unreacted -SiOH groups, these residual silanol groups are removed with a smaller silanizing reagent, such as trimethylchlorosilane, which is less sterically hindered, in a process known as *end-capping*. Currently, different methodologies are being developed such as double or triple end capping or polymeric endcapping to reduce the effect of free silanol groups on the nature of peak shapes for basic molecules.

![FIGURE-1.3. Some typical structures on the surface of a silica-based reversed phase medium](image)

The properties of a stationary phase are determined by the nature of the organosilane’s alkyl group. If R is a polar functional group, then the stationary phase will be polar. Examples of polar stationary phases include those for which R contains a cyano (–C₂H₅CN), diol (–C₃H₆OCH₂CHOHCH₂OH), or amino (–C₃H₆NH₂) functional group. The most common nonpolar stationary phases use an organochlorosilane for which the R group is an *n*-octyl (C8) or *n*-octyldecyl (C18) hydrocarbon chain. Silica-based bonded
phases are not recommended for use in aqueous solutions that have pH values above about pH 7.5. The typical structures on the surface of a silica-based reversed phase medium are shown in Figure-1.3.

Moreover, hydrolysis of the silica matrix occurs at any pH value, and bonded phases ultimately will be degraded by aqueous mobile phases, the rate being enhanced at high salt concentrations and in the presence of some ionpairing reagents. Bonded phases based on dimethylalkyl ligands are commonly regarded to be less stable at low pH-values. The quality of the silica, the ligand density, and the ligands themselves all play an important role. The hydrolytic stability of a bonded phase based on a di- or trifunctional silane at low pH are higher than monofunctional silane. The structure of the common attachment of difunctional and trifunctional ligands is shown in Figure 1.4.

![Monofunctional silane, Bifunctional silane, Trifunctional silane](image)

**FIGURE-1.4.** Structure of the common attachment of monofunctional, difunctional and trifunctional ligands.

Polystyrene-divinylbenzene (PS-DVB) base materials are stable over the whole pH range 0 to 14. These polymers are rigid, macroporous structures with pore sizes of approximately 8 nm. Aluminas of suitable particulate geometry and pore structure can be polymer-coated by crosslinking of a polybutadiene. Such stationary support phases have excellent separation efficiency and exhibit reversed-phase retention behavior.
Stationary phases used in Research work:

The non-polar (C2, C8, and C18) and polar stationary phases (Cyano and Phenyl) used in HPLC method development for the research work. The non-polar hydrocarbonaceous moieties of low polarity are ethyl (or butyl), octyl (C8), and octadecyl (C18) linear alkanes. The octadecyl packing is used when maximum retention is required and has unique selectivity. By contrast, the ethyl group is used in applications that involve very strongly retained solutes. Octyl packings are a good compromise for the separation of samples with wide-ranging polarities.

The selection of an appropriate separation column is highly dependent upon the solutes to be separated. The sample components may also play an important role for the decision. The selectivity and capacity are the most important characteristics when choosing a column stationary phase. The stationary phases with column brands used in method development for impurity profiling study are as follows;

(i) Octyl (C8) Packing: The general description of Octyl packing columns which were tested in the method development of API’s studies are discussed below:

*Zorbax XDB C8 (250 x 4.6mm ID, 5µm and 150 x 4.6mm ID, 5µm)*: Zorbax Eclipsed XDB column specifically designed to extend column life and it provide excellent peak shape for basic compounds in the pH range 6-9. As well as it gives outstanding performance for acidic compounds. Hence Zorbax XDB columns of different lengths were used in method development of APIs.

*Inertsil C8 (250 x 4.6mm ID, 5µm and 150 x 4.6mm ID, 5µm)*: It can allow operating at significantly lower back pressures than other competitive columns, without sacrificing...
efficiency. Due to Inertsil’s wide $p$H compatibility and excellent stability at elevated temperatures it was chosen for method development purpose.

**YMC-Pack C8** (150 x 4.6mm ID, 5µm): Compared to C18 phases, retention times for hydrophobic molecules is shorter on C8 material due to the reduced carbon load (10%). YMC-Pack C8 is ideally suited for the separation of many compounds which are too strongly retained on C18 phases or require greater retention than provided by C4 materials. It is one of the most versatile reversed phase materials. Hence it was considered suitable for the development of new methods.

**Novapak C8** (250 x 4.6mm ID, 5µm): Compared to C18 phases, retention times for hydrophobic molecules is shorter on C8 material due to the reduced carbon load (10%). Nova-Pack C8 is ideally suited for the separation of many compounds which are too strongly retained on C18 phases or require greater retention than provided by C4 materials. It is one of the most versatile reversed phase materials. Hence it was considered for the development of new methods.

**ii) Octadecyl (C18) Packing:** The general description of Octadecyl packing columns which were tested in the method development of API studies are discussed below:

**Zorbax XDB C18** (250 x 4.6mm ID, 5µm and 150 x 4.6mm ID, 5µm): Zorbax Eclipsed XDB are designed to provide good peak shape for basic, acidic and neutral compounds over the $p$H range 2-9. Hence Zorbax XDB columns of different lengths were tested in method development of active pharmaceutical ingredients.

These packing are used in separations involving hydroxy, esters, nitro compounds, double-bond isomers, and ring compounds that differ in double-bond content. Hence this column used for method development of API discussed in Chapter-1.
Inertsil ODS 3V (250 x 4.6mm ID, 5µm and 150 x 4.6mm ID, 5µm): It can allow operating at significantly lower back pressures than other competitive columns, without sacrificing efficiency. This column is very rugged to use and mainly this column operates at pH range 2 to 7.5.

YMC-Pack C18 (150 x 4.6mm ID, 5µm): Compared to C8 phases, retention time for hydrophobic molecules are longer on C18 material due to the reduced carbon load (15%). YMC-Pack C18 is ideally suited for the separation of many compounds which are too closely eluted on C8 phases or require greater retention than provided by C4 materials. It is one of the most versatile reversed phase materials. Hence it was considered for the development of new methods.

iii) Cyano (CN) Packing: Interacts with polar functional groups, allowing its use in both reversed-phase and normal phase chromatography.

Zorbax SB-CN (250 x 4.6mm ID, 5µm): This column is used in HPLC method development of API in research work. It is a micro-particulate column packing used for RP-HPLC & NP-HPLC. This column was tested in the method development of API. This stable bond packing is made by chemically bonding a sterically-protected cyanopropyl stationary phase to a specially prepared, ultra-high purity Zorbax Rx-SIL porous silica microsphere. The thickly covered, sterically protected, diisopropyl cyanopropyl stationary phase is chemically stable at low pH (pH < 4) and gives longer column life. This column packing have some solubility in pH>6 aqueous mobile phases. The Zorbax SB-CN provides better chromatographic performance for basic compounds. This column provides better separation of polar and non-polar compounds within the short time. Hence this column was chosen for method development of API.
iv) Phenyl Packing: Exhibits a more polar nature than either the C18 or C8, the π-electron clouds providing sites of interaction for a variety of aromatic (ring) analytes.

Zorbax SB-Phenyl (250 x 4.6mm ID, 5µm): This column is used in HPLC method development of API in research work. It is a micro particulate column packing used for RP-HPLC. This stable bond packing is made by chemically bonding a sterically-protected phenethyl stationary phase to a specially prepared, ultra-high purity, porous silica microsphere. The thickly covered, sterically protected, diisopropyl phenyl ethyl stationary phase is chemically stable at low pH (pH< 4) and gives longer column life. It is stable reverse phase packing that can be used for basic, neutral and acidic compounds. This column provides better separation of polar and non-polar compounds within the short time. The phenyl group is used for separations that rely on the interaction of aromatic compounds with the stationary phase. This column provides better separations especially for ethers, esters, nitro compounds, double-bond isomers and ring compounds that differ in double-bond content. Hence this column was tested in method development of API.

1.2.1.3 Mobile phases in HPLC

An initial goal of method development is to obtain adequate retention of all the analytes. After stationary phase selection, the proper selection of the mobile phase is the second most important step in the development of the separation method. The main requirement for the mobile phase is that it has to dissolve the analytes up to the concentration suitable for the detection. Type of mobile phase used may have a big effect on the retention. It can help or suppress an ionization of the analyte molecules.
Variation of the solvents and buffers in mobile phase composition provides the great flexibility of HPLC separations.

Basically, the elution order of solutes depends on the polarity index of mobile phase solvents and buffers. In a reverse-phase (RP) separation the more polar solute spends proportionally less time in the non-polar stationary phase and is the first solute to elute from the column. Retention times are controlled by selecting the mobile phase, with a more polar mobile phase leading to longer retention times. If, for example, a separation is poor because the solutes are eluting too quickly, switching to a more polar mobile phase leads to longer retention times and more opportunity for an acceptable separation. When two solutes are adequately resolved, switching to a less polar mobile phase may provide an acceptable separation with a shorter analysis time.

In a normal-phase (NP) separation the order of elution is reversed, with the least polar solute being the first to elute. Decreasing the polarity of the mobile phase leads to longer retention times, whereas shorter retention times require a mobile phase of higher polarity. The polarity index of solvents is shown in Table-1.1. Selection of mobile phase composition is dependent on the type of solutes. The properties of organic solvents, buffer and ion-pairing reagents are discussed below.\textsuperscript{29-32}

1.2.1.3.1 Organic modifier

In reversed phase chromatography the organic solvent (modifier) is added to decrease the polarity of the aqueous mobile phase. As the polarity of the mobile phase decreases, it’s eluting strength increases. Although a large variety of organic solvents can be used in reversed phase chromatography, in practice only a few are routinely employed. The two most widely used organic modifiers are acetonitrile (ACN) and methanol
(MeOH), although acetonitrile is the more popular choice. Tetrahydrofuran (THF) is stronger than ACN, which in turn is stronger than MeOH. THF yields different selectivity in RPLC but its use is not very popular due to its toxicity and its tendency to form peroxide. Isopropanol (2-propanol) can be employed because of its strong eluting properties, but is limited by its high viscosity which results in lower column efficiencies and higher backpressures. Both acetonitrile and methanol are less viscous than isopropanol. Table-1.1 shows that methanol, acetonitrile and isopropanol are essentially UV transparent. This is a crucial property for reversed phase chromatography since column elution is typically monitored using UV detectors.

**TABLE-1.1. Properties of common chromatographic mobile phases**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Refractive Index</th>
<th>Polarity Index</th>
<th>UV Cut off</th>
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<tbody>
<tr>
<td>Cyclohexane</td>
<td>1.423</td>
<td>0.04</td>
<td>210</td>
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<tr>
<td>n-Hexane</td>
<td>1.372</td>
<td>0.1</td>
<td>210</td>
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<tr>
<td>Carbon tetrachloride</td>
<td>1.457</td>
<td>1.6</td>
<td>265</td>
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<tr>
<td>Isopropyl ether</td>
<td>1.365</td>
<td>2.4</td>
<td>220</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.494</td>
<td>2.4</td>
<td>286</td>
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<tr>
<td>Diethyl ether</td>
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<td>1,4 Dioxane</td>
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<td>4.8</td>
<td>215</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>1.411</td>
<td>5.0</td>
<td>205</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.326</td>
<td>5.1</td>
<td>210</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.341</td>
<td>5.8</td>
<td>190</td>
</tr>
<tr>
<td>Water</td>
<td>1.333</td>
<td>10.2</td>
<td>190</td>
</tr>
</tbody>
</table>
Acetonitrile is used almost exclusively when separating non-polar impurities from each other and from API. Most API’s only absorb at low wavelengths in the ultra-violet spectrum (typically less than 225 nm) and acetonitrile provides much lower background absorbance than other common solvents at low wavelengths. The retention or capacity factor \((k')\), for a given solute is a function of the mobile phase polarity. The elution order can be affected by changing the type of organic modifier or by the addition of ion pairing agents.

1.2.1.3.2 Buffer

Buffers are commonly used to control the \(pH\) of the mobile phase for the separation of acidic and basic analytes. The retention of API and its related impurities in reversed phase chromatography can be modified by mobile phase \(pH\). It depends on their hydrophobicity (ionisable groups present in solutes). The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic and, therefore, its retention decreases. The degree of ionisation will depend on the \(pH\) of the mobile phase. Acids lose a proton and become ionized when \(pH\) increases and bases gain a proton and become ionized when \(pH\) decreases. (See Figure-1.5) Therefore, when separating mixtures containing acids and/or bases by reversed phase HPLC, it is necessary to control the \(pH\) of the mobile phase using an appropriate buffer in order to achieve reproducible results. The stability of silica-based reversed phase media dictates that the operating \(pH\) of the mobile phase should be below \(pH\) 7.5. The amino groups contained in API and its related impurities are charged below \(pH\) 7.5. The carboxylic acid groups, however, are neutralised as the \(pH\) is decreased. The mobile phase used in reversed phase chromatography is generally prepared with strong acids such as trifluoroacetic acid (TFA)
or ortho-phosphoric acid. These acids maintain a low pH environment and suppress the ionization of the acidic groups in the solute molecules. Varying the concentration of strong acid components in the mobile phase can change the ionization of the solutes and, therefore, their retention behavior.

For the most robust methods, it is recommended that separations be developed at a mobile phase pH where the retention of analytes is little affected by changes in pH. When separating bases, for example, acidic mobile phases usually show better reproducibility than neutral mobile phases.

An acceptable separation of ionic compounds can only be achieved at a mobile phase pH where retention and resolution are sensitive to pH. In fact, mobile phase pH can be a powerful variable to use to adjust retention and achieve separations of mixtures containing ionic compounds.

**FIGURE-1.5.** The Effect of pH on the Retention of Acids and Bases in RP-HPLC
TABLE-1.2. Commonly Used Buffers (their pKₐ and UV cutoffs) for RP-HPLC

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pKa</th>
<th>Buffer Range</th>
<th>UV Cutoff (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>2.1</td>
<td>1.1-3.1</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>6.2-8.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.3</td>
<td>11.3-13.3</td>
<td></td>
</tr>
<tr>
<td>Formic acid¹</td>
<td>3.8</td>
<td>2.8-4.8</td>
<td>210</td>
</tr>
<tr>
<td>Acetic acid¹</td>
<td>4.8</td>
<td>3.8-5.8</td>
<td>210</td>
</tr>
<tr>
<td>Trifluoroacetic acid¹</td>
<td>0.3</td>
<td>-</td>
<td>210</td>
</tr>
<tr>
<td>Ammonium¹</td>
<td>9.2</td>
<td>8.2 – 10.2</td>
<td>200</td>
</tr>
<tr>
<td>Carbonate¹</td>
<td>6.4</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>3.1</td>
<td>2.1-4.1</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>3.7-5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>4.4-6.4</td>
<td></td>
</tr>
<tr>
<td>Tris (hydroxymethyl) aminomethane</td>
<td>8.3</td>
<td>7.3-9.3</td>
<td>210</td>
</tr>
<tr>
<td>1-Methylpiperidine</td>
<td>10.1</td>
<td>9.1 – 11.1</td>
<td></td>
</tr>
<tr>
<td>Triethylamine¹</td>
<td>11.0</td>
<td>10.0-12.0</td>
<td>200</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>11.3</td>
<td>10.3-12.3</td>
<td>200</td>
</tr>
</tbody>
</table>

¹Volatile buffer that are MS compatible

Normally, in mobile phase optimum buffering capacity occurs at a pH equal to the pKₐ of the buffer. Most of the buffers only effective within ±1 pH unit from its pKₐ, hence thoughtful selection of the proper buffer within its buffering range is preferable for separation of compounds. Beyond that, buffering capacity will be inadequate. Above Table-1.2 lists the some commonly used buffers for RP-HPLC with its respective information of pKₐ values, optimal range for controlling pH, and uv cutoffs.

1.2.1.3.3 Ion pairing agents

The retention times of solutes such as API and its related impurities can be modified by adding ion pairing agents to the mobile phase. Ion pairing agents bind to the
solute by ionic interactions and it increase the hydrophobicity of the solute molecule and change selectivity. Both anionic and cationic ion pairing agents are used depending on the ionic character of the solute molecule and the pH of the mobile phase. Long-chain alkyl sulfonates are commonly used for the separation of water-soluble basic analytes. Hexanesulfonate binds with basic analytes to form ion-pairs. These neutral ion-pairs are now more hydrophobic than their unassociated protonated basic functional group resulting in higher retention times. An ion-pairing reagent provides an additional parameter to facilitate resolution of basic analytes from other components in the sample. The amine modifiers such as triethylamine (TEA) are often added in the mobile phase to reduce peak tailing caused by the strong interaction of basic analytes with acidic surface silanols. For acidic analytes, ion-pairing reagents such as tetraalkylammonium salts are often used. The concentration of ion pairing agents in the mobile phases is generally in the range 0.1-0.3%. Potential problems include possible absorbance of UV light by the ion pairing agent and changes in extinction coefficient with concentration of organic modifier. This can result in either ascending or descending baselines during gradient elution. The name of some common anionic ion pairing agents are as; trifluoroacetic acid, pentafluoropropionic acid, heptafluorobutyric acid, ammonium acetate and phosphoric acid and cationic ion pairing agents are as; tetramethylammonium chloride, tetrabutylammonium chloride and triethylamine.

The selected mobile phase in HPLC refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution. A sample solution is injected into the mobile phase of an assay through the injector port. As a sample solution flows through a column with the mobile phase, the
components of that solution migrate according to the non-covalent interactions of the compound with the column. The chemical interactions of the mobile phase and sample, with the column, determine the degree of migration and separation of components contained in the sample. For example, those samples which have stronger interactions with the mobile phase than with the stationary phase will elute from the column faster and thus have a shorter retention time, while the reverse is also true. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

1.2.2 Gas Chromatography (GC)

Gas Chromatography (GC) is a commonly used analytic technique in many research and industrial laboratories. A broad variety of samples can be analyzed as long as the compounds are sufficiently thermal stable and volatile enough. GC is a very powerful and one of the most common instrumental analysis techniques used in many research and industrial laboratories. It provides both qualitative and quantitative information about individual components in a sample or compound. GC involves separating the different components in a sample from each other. This allows the easy identification and measurement of the individual components in a sample. The components are separated primarily by the differences in their volatilities and structures. Many components and samples are not suitable for gas chromatographic analysis due to their physical and chemical properties. GC mostly applied for the analysis of chromophoric and non-chromophoric components that can be easily vaporized.

For a sample component to be suitable for GC analysis, it must possess appreciable volatility at temperatures below 350-400°C. Another characteristic is the compound must
be thermally stable and be rapidly transformed into a vapor without degradation or reacting with other compounds. This type of information about a compound is not readily available in references and other sources. However, the partial information estimates and generalizations can be made from the structure of the compounds. The compound structure, its boiling point and molecular weight can be used as indicators of potential GC analysis suitability. Non-volatile compounds are not suitable for GC analysis because they do not readily vaporize. Boiling point of compounds are not always good indicators of volatility. There are many high boiling compounds that can be analyzed by GC. As a general criteria, the greater the molecular weight or polarity of a compound, the lower its volatility. Both factors have to be considered. For example, a large, non-polar compound may be more volatile than a small, polar compound. Also, one polar group on a large molecule has less of an influence than one polar group on a small molecule. Hydrocarbons with molecular weights over 500 are routinely analyzed using standard GC systems, and hydrocarbons with molecular weights over 1400 have been easily analyzed using the properly equipped GC and type of column. The presence of polar functionalities such as hydroxyl and amine groups severely decreases compound volatility. Some small molecules such as sugars and amino acids can not be easily analyzed by GC due to the large number of polar groups. As a rule, inorganic compounds are not suitable for GC analysis. Metals and salts do not possess the required volatility. Many organometallics have sufficient volatility for analysis due to the high organic content of these molecules. Most organic compounds are suitable for GC analysis; however, there are many exceptions. Many biomolecules and pharmaceuticals are thermally sensitive and degrade at the temperatures used in GC. Some compounds react with the materials used in various parts of GC and can
not successfully analyzed by GC. There are no realistic, absolute guidelines that can be used to determine whether a compound can be analyzed by GC. Overall, it has been estimated that only about 10% of all compounds can be analyzed by GC.

1.2.2.1 The Basic Parts of a Gas Chromatograph (GC)

The GC is composed of several components. These components include the carrier gas supply and flow controller, injector, oven, column, detector, and a data system.\textsuperscript{33-35} A schematic diagram of a typical GC is shown in Figure-1.6.

![FIGURE-1.6. Basic instrumentation of gas chromatography.](image)

The analysis starts when a small quantity of sample, which may be a gas or liquid, is injected into a stream of an inert gaseous mobile phase (often called the carrier gas). Here analytes are vaporized and mixed uniformly in the mobile phase. The vaporized sample is swept into a packed or capillary column where the sample’s components separate based on their ability to distribute themselves between the mobile and stationary phases. The column containing stationary phase is situated in a variable temperature oven. At the
end of column the mobile phase passes through a detector. The signal is appears on the chart as a plot of time versus the composition of the carrier gas stream. The details of GC instrumentation parts are as discussed below.

**1.2.2.1 Carrier gas and flow regulation**

High purity gases are supplied from a pressurized cylinder or gas generator. Pressure regulators on the cylinders or generators control the amount of gas delivered to the gas chromatograph. Flow controllers or pressure regulators in the GC control the flow of the various gases once they enter the instrument. The column is installed between the injector and detector. Gas at a precisely controlled flow is supplied to the injector; this gas is called the carrier gas (mobile phase). The carrier gas flows through the injector and into the open tubular column. The most common carrier gases for GC are He, Ar, and N₂, which have the advantage of being chemically inert toward both the sample and the stationary phase. The choice of carrier gas to use is often decided by the instrument’s detector. The carrier gas travels the length of the column and exits through a detector. To function as desired, most detectors require specific gases at the proper flow rates.

**1.2.2.1.2 Injector**

The injector introduces the sample into the open tubular column. The injector is a hollow, metal cylinder containing a glass liner. The column is inserted into the bottom of the injector. A liquid, or sometimes a gas, is introduced into the injector through a septum using a small syringe. The injector is heated to 100-300°C, thus any volatile sample components are rapidly transformed into a vapor. The carrier gas mixes with the vaporized portion of the sample and carries the sample vapors into the column.
An on-column injector deposits the sample directly into the column without a vaporization step and it is used for select types of samples. In some cases, nonsyringe techniques utilizing specialized equipment or devices (e.g., purge and trap, headspace, and valves) can be used to introduce a sample into a column.

### 1.2.2.1.3 Capillary Column and Oven

The column exists in an oven whose temperature is accurately controlled. If unimpeded, vaporized compounds move through the column at the same rate as the flowing carrier gas. However, the interior walls of columns are coated with a thin film of polymeric material called the stationary phase. This stationary phase impedes the movement of each component down the column by a different amount. This behavior is called retention. The length and diameter of the column, the chemical structure and amount of the stationary phase, and the column temperature all affect component retention. If all of these factors are properly selected, each component travels through the column at a different rate. This makes the components exit the column at different times. As each component leaves the column, its presence and amount are measured by the detector.

### 1.2.2.1.4 Detector

As each component exits the column, it enters the detector. The detector interacts with the components based on some physical or chemical property. There are many detectors which can be used in GC. Some detectors respond to every component while others respond only to a select group of components. The interaction generates an electrical signal which is related to the rate at which solute molecules enter the detector. The detector signal is then sent to a recording device for plotting.
The GC Detectors are grouped into two types viz., concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not destroy the sample. Dilution of with make-up gas will lower the detectors response. Thermal conductivity (TCD), Electron capture (ECD), Photo-ionization (PID) are the example of concentration dependant type of detectors. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. Flame ionization (FID), Mass spectrometer (MS), Nitrogen-phosphorus (NPD), Flame photometric (FPD), Hall electrolytic conductivity detectors are the example of mass flow dependant type of detectors.

1.2.2.1.5 Data System

The recording device plots the size of the detector signal versus the time elapsed since sample introduction into the injector. The plot is called a chromatogram and appears as a series of peaks. The most common data recording devices are computer (PC) based. PC based data system are extremely powerful and offer numerous data plotting, reporting and storage options, thus their popularity. Most computer data system can also control and automate the operation of the GC.

1.2.2.2 Brief discussion on stationary phases used in GC for research work.

The selection of an appropriate stationary phase can also help to improve the efficiency of method development in GC analysis. An optimized chromatographic separation begins with the column. The selections of the proper capillary column in research work for GC analysis are based on four significant factors: stationary phase,
column I.D., film thickness, and column length. Selectivity in GC is influenced by the choice of stationary phase. Elution order in GLC is determined primarily by the solute’s boiling point and, to a lesser degree, by the solute’s interaction with the stationary phase. Solutes with significantly different boiling points are easily separated. On the other hand, two solutes with similar boiling points can be separated only if the stationary phase selectively interacts with one of the solutes. In general, non-polar solutes are more easily separated with a non-polar stationary phase, and polar solutes are easier to separate using a polar stationary phase. The main criteria for selecting a stationary phase are that it should be chemically inert, thermally stable, of low volatility, and of an appropriate polarity for the solutes being separated.

Non-polar solutes are generally composed only of carbon and hydrogen atoms and contain carbon-carbon single bonds. Normal hydrocarbons (n-alkanes) are the most common non-polar solutes analyzed by capillary GC. Polar components are composed primarily of carbon and hydrogen atoms, but also contain one or more atoms of bromine, chlorine, fluorine, nitrogen, oxygen, phosphorus, or sulfur. Alcohols, amines, carboxylic acids, diols, esters, ethers, ketones, and thiols are typical polar components analyzed by capillary GC. The common typical stationary phases are polysiloxane and polyethylene glycol and which were used for method development of some API’s, as discussed in Chapter-1 and Chapter-6. The details of these stationary phases are as follows;

**Polysiloxanes:**

Polysiloxanes are the most common stationary phases. They are available in the greatest variety and are the most stable, robust and versatile. The most basic polysiloxane is the 100% methyl substituted. When other groups are present, the amount is indicated as the
percent of the total number of groups. For example, DB-5; a 5% diphenyl-95% dimethyl polysiloxane contains 5% phenyl groups and 95% methyl groups. The "di-" prefix indicates that each silicon atom contains two of that particular group. Sometimes this prefix is omitted even though two identical groups are present. If the methyl percentage is not stated, it is understood to be present in the amount necessary to make 100% (e.g., 50% phenyl-methyl polysiloxane contains 50% methyl substitution). Typical trade name of this type of column is OV-17, DB-17, SPB-7, BP-10, HP-17, RTx-17 and AT-50.

Cyanopropylphenyl percent values can be misleading. A 14% cyanopropylphenyl-dimethyl polysiloxane contains 7% cyanopropyl and 7% phenyl (along with 86% methyl). The cyanopropyl and phenyl groups are on the same silicon atom, thus their amounts are summed. Typical trade name of this type of column is HP-1301, DB-624.

**Polyethylene Glycols:**

Polyethylene glycols (PEG) are widely used as stationary phases. Stationary phases with "wax" or "FFAP" in their name are some type of polyethylene glycol (e.g. DB-Wax, Supelsowax-10 and Stabilwax). Polyethylene glycols stationary phases are not substituted, thus the polymer is 100% of the stated material. These are less stable, less robust and have lower temperature limits than most polysiloxanes. With typical use, these exhibit shorter lifetimes and are more susceptible to damage upon over heating or exposure to oxygen. The unique separation properties of polyethylene glycol make these liabilities tolerable. Polyethylene glycol stationary phases must be liquids under GC temperature conditions.
1.3 Key factors to consider during stability indicating method development

In an early phase development there is limited knowledge about the chemistry of the new drug substances with respect to synthetic impurities and degradation pathways. It is, therefore, desirable to develop suitable chromatographic methods that show applicability to a broad range of potential impurities and degradation products. The methods are intended to provide the information necessary to guide the improvement of a synthesis route or a new drug formulation. During product process-development, a systematic stability study plan is critical, as it can affect the product life cycle and project planning. Before the drug product is available, stress testing (a forced degradation study) of the drug substance under different conditions can help to identify possible degradation products, the degradation pathway, and the stability of the drug substance.

As a starting point to method development, the physicochemical properties of the API such as structure(s), solubility/stability in different solvents, pKa(s), spectra (UV, IR, MS, and NMR), and chirality should be determined. It is also extremely valuable to know the targeted potency (if known), the synthetic routes used in production (to postulate potential synthetic impurities), any information regarding intended formulations, and to review any literature documenting the analyses of similar compounds.

One main purpose in initiating method development is to generate samples that can be used for method development. It is highly desirable to obtain source materials containing process impurities that can serve as markers for positive identification. If authentic process impurities or synthetic intermediates are unavailable, mother liquors or reaction mixtures can be utilized. Crude batches that have not yet undergone final crystallization, or any other batch containing a large number of process-related impurities, are also ideal for use
in tracking relevant impurities/degardants. Ideally, the samples should collectively contain the API, all significant synthetic impurities and degradation products. The synthetic impurities are typically obtained using early batches of drug substance, along with critical intermediates and starting materials. The $\lambda_{\text{max}}$ value of discovered impurities (Process as well as degradation) and API should be determined. It guides for which type of techniques should be used for their identification and determination. For example, if compound is UV inactive i.e non-chromophoric it can be determined by GC and / or HPLC-RI, TLC techniques, and if compound is UV active i.e. chromophoric it can be determined by HPLC-PDA technique.

A general way to obtain samples of degradation products is to place the drug substances or an early formulation under a variety of stress conditions. In general, the drug substances are subjected to acidic, neutral, and basic solutions, as well as to high temperatures, light and oxidative conditions (hydrogen peroxide solution). A recent review of approaches to conducting forced decomposition studies shows a large variability in the experimental approach within the pharmaceutical industry. The practical aspects related to stress testing are neither addressed by regulatory guidelines, nor by any other document. Therefore, a critical study was done of the reports in literature for the stress conditions used for determining inherent stability of new drug molecules. Most useful information was found in the monographs given in the volumes of “Analytical Profiles of Drug Substances and Excipients”.

The monographs carry a stability section which records the inherent stability behavior of the drug and the conditions employed to determine the same. Examples of drugs where complete information with respect to strength of reactant, temperature of study, time period of exposure and extent of decomposition was available
were picked up. The mention of use of stress conditions was also found in some reports in literature on establishment of stability-indicating assays. The total information about the different types of reactivities, viz., hydrolysis in acid, alkaline and oxidation was tabulated in Table 1.3-1.5.

**TABLE-1.3.** Selected examples of stress conditions used for hydrolysis of drugs in acid

<table>
<thead>
<tr>
<th>Drug</th>
<th>Strength</th>
<th>Reaction conditions / Temperature</th>
<th>Time</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>0.1 N HCl</td>
<td>RT</td>
<td>24 h</td>
<td>No degradation</td>
<td>39</td>
</tr>
<tr>
<td>Valacyclovir</td>
<td>0.1 M HCl</td>
<td>RT</td>
<td>8 h</td>
<td>Considerable degradation</td>
<td>40</td>
</tr>
<tr>
<td>Ranitidine Hydrochloride</td>
<td>0.1 N HCl</td>
<td>60°C, Reflux</td>
<td>30 min.</td>
<td>11.3 % Degradation</td>
<td>41</td>
</tr>
<tr>
<td>Eplerenone</td>
<td>0.1 M HCl</td>
<td>60°C</td>
<td>6 h</td>
<td>22 % Degradation</td>
<td>42</td>
</tr>
<tr>
<td>Ethacrynic acid</td>
<td>0.1 N HCl</td>
<td>60°C</td>
<td>21 days</td>
<td>71.6 % Degradation</td>
<td>43</td>
</tr>
<tr>
<td>Rimonabant</td>
<td>0.5 M HCl</td>
<td>60°C</td>
<td>48 h</td>
<td>No degradation</td>
<td>44</td>
</tr>
<tr>
<td>Tazarotene</td>
<td>0.5 N HCl</td>
<td>60°C</td>
<td>48 h</td>
<td>No degradation</td>
<td>45</td>
</tr>
<tr>
<td>Brimonidine Tartrate</td>
<td>1 M HCl</td>
<td>60°C, Reflux</td>
<td>24 h</td>
<td>No degradation</td>
<td>46</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>1 M HCl</td>
<td>80°C</td>
<td>8 h</td>
<td>Considerable degradation</td>
<td>10 % Degradation</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>1 N HCl</td>
<td>85°C</td>
<td>25 min.</td>
<td>10 % Degradation</td>
<td>48</td>
</tr>
<tr>
<td>Balsalazide Disodium</td>
<td>1 M HCl</td>
<td>100°C</td>
<td>4 h</td>
<td>20 % Degradation</td>
<td>49</td>
</tr>
<tr>
<td>Montelukast Sodium</td>
<td>1 M HCl</td>
<td>RT</td>
<td>15 min.</td>
<td>20 % Degradation</td>
<td>50</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>2 N HCl</td>
<td>100°C</td>
<td>2 h</td>
<td>4 % Degradation</td>
<td>51</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>2 M HCl</td>
<td>60°C</td>
<td>48 h</td>
<td>No degradation</td>
<td>52</td>
</tr>
<tr>
<td>Felbamate</td>
<td>3 M HCl</td>
<td>80°C, Reflux</td>
<td>3 h</td>
<td>9 % Degradation</td>
<td>53</td>
</tr>
<tr>
<td>Cilastazole</td>
<td>1 N, 2 N and 5 N HCl</td>
<td>60°C, Reflux</td>
<td>5 h</td>
<td>No degradation</td>
<td>54</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>5 M HCl</td>
<td>60°C</td>
<td>48 h</td>
<td>8 % Degradation</td>
<td>55</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>5 N HCl</td>
<td>80°C</td>
<td>6 h</td>
<td>Negligible degradation</td>
<td>56</td>
</tr>
</tbody>
</table>
TABLE-1.4. Selected examples of stress conditions used for hydrolysis of drugs in alkali

<table>
<thead>
<tr>
<th>Drug</th>
<th>Strength</th>
<th>Reaction conditions / Temperature</th>
<th>Time</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valacyclovir</td>
<td>0.01 M NaOH</td>
<td>RT</td>
<td>8 h</td>
<td>Considerable degradation</td>
<td>40</td>
</tr>
<tr>
<td>Eplerenone</td>
<td>0.01 M NaOH</td>
<td>RT</td>
<td>1 h</td>
<td>30 % Degradation</td>
<td>42</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>0.01 M NaOH</td>
<td>40°C</td>
<td>8 h</td>
<td>Considerable degradation</td>
<td>47</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>0.05 N NaOH</td>
<td>RT</td>
<td>1.5 h</td>
<td>10 % Degradation</td>
<td>48</td>
</tr>
<tr>
<td>Ranitidine Hydrochloride</td>
<td>0.1 M NaOH</td>
<td>60°C, Reflux</td>
<td>30 min.</td>
<td>12.4 % Degradation</td>
<td>41</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>0.1 M NaOH</td>
<td>RT</td>
<td>24 h</td>
<td>No degradation</td>
<td>39</td>
</tr>
<tr>
<td>Felbamate</td>
<td>0.3 M NaOH</td>
<td>RT</td>
<td>0.5 h</td>
<td>8 % Degradation</td>
<td>53</td>
</tr>
<tr>
<td>Tazarotene</td>
<td>0.5 N NaOH</td>
<td>60°C</td>
<td>48 h</td>
<td>4 % Degradation</td>
<td>45</td>
</tr>
<tr>
<td>Ritonamabt</td>
<td>0.5 M NaOH</td>
<td>60°C</td>
<td>48 h</td>
<td>No degradation</td>
<td>44</td>
</tr>
<tr>
<td>Brimonidine Tartrate</td>
<td>1 M NaOH</td>
<td>60°C, Reflux</td>
<td>24 h</td>
<td>No degradation</td>
<td>46</td>
</tr>
<tr>
<td>Balsalazide Disodium</td>
<td>1 M NaOH</td>
<td>100°C</td>
<td>1 h</td>
<td>25 % Degradation</td>
<td>49</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>1 N NaOH</td>
<td>80°C</td>
<td>1 h</td>
<td>5 % Degradation</td>
<td>51</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>2 M NaOH</td>
<td>60°C</td>
<td>48 h</td>
<td>3 % Degradation</td>
<td>52</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>5 N NaOH</td>
<td>80°C</td>
<td>6 h</td>
<td>5 % Degradation</td>
<td>56</td>
</tr>
<tr>
<td>Montelukast Sodium</td>
<td>5 M NaOH</td>
<td>85°C</td>
<td>2 h</td>
<td>No degradation</td>
<td>50</td>
</tr>
</tbody>
</table>
TABLE-1.5. Selected examples of stress conditions used for oxidation of drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Strength</th>
<th>Reaction conditions / Temperature</th>
<th>Time</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitidine Hydrochloride</td>
<td>0.1% H₂O₂</td>
<td>60°C, Reflux</td>
<td>30 min.</td>
<td>7.9 % Degradation</td>
<td>41</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>3% H₂O₂</td>
<td>60°C</td>
<td>1 h</td>
<td>6 % Degradation</td>
<td>55</td>
</tr>
<tr>
<td>Tazarotene</td>
<td>3% H₂O₂</td>
<td>60°C</td>
<td>1 h</td>
<td>5 % Degradation</td>
<td>45</td>
</tr>
<tr>
<td>Linezolid</td>
<td>3% H₂O₂</td>
<td>RT</td>
<td>1 h</td>
<td>Considerable Degradation</td>
<td>39</td>
</tr>
<tr>
<td>Valacyclovir</td>
<td>3 % H₂O₂</td>
<td>RT</td>
<td>7 days</td>
<td>Considerable degradation</td>
<td>40</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>3% H₂O₂</td>
<td>60°C</td>
<td>1 h</td>
<td>Negligible degradation</td>
<td>56</td>
</tr>
<tr>
<td>Montelukast Brimonidine Tartrate</td>
<td>3 % H₂O₂</td>
<td>RT</td>
<td>10 min</td>
<td>13 % Degradation</td>
<td>50</td>
</tr>
<tr>
<td>Balsalazide Disodium</td>
<td>6% H₂O₂</td>
<td>RT</td>
<td>48 h</td>
<td>2% Degradation</td>
<td>46</td>
</tr>
<tr>
<td>Rimonabant</td>
<td>6% H₂O₂</td>
<td>100°C</td>
<td>4 h</td>
<td>30 % Degradation</td>
<td>49</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>6% H₂O₂</td>
<td>60°C</td>
<td>48 h</td>
<td>11 % Degradation</td>
<td>52</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>6% H₂O₂</td>
<td>60°C</td>
<td>1 h</td>
<td>6 % Degradation</td>
<td>55</td>
</tr>
<tr>
<td>Felbamate</td>
<td>10% H₂O₂</td>
<td>Refluxed at 80°C</td>
<td>48 h</td>
<td>No degradation</td>
<td>53</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>20% H₂O₂</td>
<td>RT</td>
<td>24 h</td>
<td>No degradation</td>
<td>47</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>30% H₂O₂</td>
<td>RT</td>
<td>24 h</td>
<td>Complete degradation</td>
<td>50</td>
</tr>
<tr>
<td>Eplerenone</td>
<td>30 % H₂O₂</td>
<td>60°C</td>
<td>6 h</td>
<td>26 % Degradation</td>
<td>42</td>
</tr>
</tbody>
</table>

In each table, the drugs were listed in an order of exaggeration of the strength of the reactant. The stress conditions used for the study of decomposition in acid conditions revealed that hydrochloric acid at strength of 0.1 N-5 N was mostly used. Large variations were also seen in the reaction (temperature) conditions and periods of study. The
temperature range varied between room temperature and 100°C. Examples can be seen in Table-1.3,\textsuperscript{39-51} where drugs were kept around 100°C or under refluxed conditions for periods ranging from a few minutes to as long as even 21-days. The some drugs like Cilastazole and Brimonidine tartrate shows no degradation even after refluxing in 5 N and 1 N HCl for 5 hrs and 24 hrs respectively, while others like Valacyclovir undergo considerable degradation when they are kept in 0.1 N acid for 8 h at room temperature. It is evident from Table-1.4 that stress conditions used for the hydrolysis of drugs under alkaline conditions run parallel to those used for acid conditions. Sodium hydroxide is mostly used, at strength of 0.1 N and 5 N. Just like acidic degradation, lot of variation is observed in time and temperature of exposure of drugs to alkali. Depending on the inherent stability characteristics, some drugs show no degradation even after refluxing in 0.1 N -5 N NaOH for 2-48 hrs while others like Eplerenone and Oseltamivir undergo considerable degradation when they are kept in 0.01 N and 0.05 N alkali for 1 h and 5 h respectively, at room temperature. In terms of oxidative degradation studies, hydrogen peroxide has been employed at strengths from 0.3% to 30%, as shown in Table-1.5. In some drugs extensive degradation is seen when exposed to 3% H\textsubscript{2}O\textsubscript{2} for very short time periods at room temperature (e.g., Montelukast Sodium and linezolid). In other cases, exposure to high concentrations of H\textsubscript{2}O\textsubscript{2} even under extreme conditions does not cause any significant degradation (e.g., Oseltamivir, Felbamate, etc.). The behavior is on expected lines, as some drugs are in fact oxidisable, while there are others that are not. The latter are not expected to show any change even in the presence of high dose of oxidising agents. Table-1.6 emphasizes the fact that there is a lot of variation in the manner in which stress photo stability testing is done on different drugs. Mostly the drugs are exposed to short/long
wavelength UV light, or as per photo stability ICH guideline (Q2R1). The photolability studies are done on drugs in either solid form or solution. Table-1.7 shows stress conditions employed for thermal stability of drugs during stress study, where the applied temperature for stressing of drug has been varied between 65°C to 120°C.

**TABLE-1.6.** Selected examples of stress conditions used for photolysis of drug

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conditions</th>
<th>Exposure conditions</th>
<th>Time</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brimonidine</td>
<td>UV Light, 254nm</td>
<td>60°C</td>
<td>10 days</td>
<td>No degradation</td>
<td>46</td>
</tr>
<tr>
<td>Tartrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>Sunlight</td>
<td>60,000–70,000 lux</td>
<td>2 d</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>UV Light, 254nm</td>
<td>200 W h m</td>
<td>10 days</td>
<td>No degradation</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Visible light</td>
<td>1,200 K lux</td>
<td>10 days</td>
<td>No degradation</td>
<td></td>
</tr>
<tr>
<td>Eplerenone</td>
<td>UV Light, 254nm</td>
<td>200 W h m</td>
<td>48 h</td>
<td>No degradation</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>UV Light, 366nm</td>
<td>200 W h m</td>
<td>48 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montelukast</td>
<td>Photolytic</td>
<td>10K lux/h</td>
<td>48 h</td>
<td>10 % Degradation</td>
<td>50</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE-1.7.** Selected examples of stress conditions used for thermal stability of drug

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conditions Temperature</th>
<th>Time</th>
<th>Remarks</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brimonidine</td>
<td>60°C</td>
<td>10 days</td>
<td>No degradation</td>
<td>46</td>
</tr>
<tr>
<td>Tartrate</td>
<td>100°C</td>
<td>10 days</td>
<td>No degradation</td>
<td>49</td>
</tr>
<tr>
<td>Balsalazide</td>
<td>100°C</td>
<td>10 days</td>
<td>No degradation</td>
<td>49</td>
</tr>
<tr>
<td>Disodium</td>
<td>100°C</td>
<td>10 days</td>
<td>No degradation</td>
<td>52</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>105°C</td>
<td>10 days</td>
<td>No degradation</td>
<td>55</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>120°C</td>
<td>1 h</td>
<td>22 % Degradation</td>
<td>48</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>120°C</td>
<td>1 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the information provided above, it is apparent that stress-testing conditions have varied greatly from compound to compound and from investigator to investigator.
Extremely harsh conditions have been commonly used in the past to ensure degradation, even if the conditions far exceeded plausible exposures.

More recently, several articles relevant to stress testing have appeared in the pharmaceutical literature. A paper by Singh and Bakshi in 2000 provides the most thorough collection of references to various degradation studies of drug products, documenting the diversity of conditions and approaches to stress testing. This paper attempts to provide a classification system (Extremely labile, Very labile, Labile, Stable) based on a defined systematic approach. It is not clear from the article on what basis (scientific or otherwise) the classification system was devised; however, the paper does define “endpoints” to stressing (albeit, fairly harsh endpoints), allowing for the conclusion that a particular compound may be regarded as “stable” under a certain set of conditions.

Usually the goal is to degrade the parent drug by 10% to 30%. During forced degradation study, overstressing and under stressing the sample may happen. Overstressing the sample may lead to further degradation of the primary degredants or it may generate irrelevant degredants that would never be seen in formal stability studies or during reactions. At the other extreme, under stressing may fail to generate important degradation products that may be generated in formal stability studies. The desired extent of degradation can be achieved by varying one or more of the stress conditions, e.g., exposure time, temperature, or concentration of stressing agent (acid, base, oxidizer, etc.).

For chromophoric impurities determination in API, reversed phase HPLC with PDA detector is generally the method of choice. A typical reversed-phase liquid chromatographic (RPLC) system consists of a relatively polar mobile phase and non-polar stationary phase. Water (or a buffer) is used as the weak solvent and acetonitrile, methanol,
The retention and separation of solutes are based on their partition between the two phases. Variations in silica purity, bonding chemistry, and surface chemistry (end capping) will affect selectivity of the stationary phase. Many types of RPLC columns are available, including classic C8, C18, phenyl, and cyano phases with different forms of silica (base-deactivated, high purity, etc.) as well as polar-embedded group columns. RPLC systems offer a wide range of selectivity. Generally, the traditional C8/C18 phases are less effective in separation of positional isomers than the recently marketed polar-embedded columns. The normal-phase liquid chromatographic (NPLC) system consists of a relatively non-polar mobile phase and polar stationary phase. Silica, diol, cyano, or amino bonded phases are typically used as the stationary phase and hexane (weak solvent) in combination with ethyl acetate, propanol, or butanol (strong solvent) as the mobile phase. The retention and separation of solutes are achieved through adsorption/desorption. Normal phase systems usually show better selectivity for positional isomers and can provide orthogonal selectivity compared with classical RPLC. Diluted acid or a buffer usually is needed in the mobile phase to control the pH and ensure the reproducibility of retention times. The use of RPLC is ideal for pharmaceutical analyses because of the broad range of commercially available stationary phases; because the most common RPLC mobile phases (buffers with acetonitrile or methanol) have low UV cut-off wavelengths, which facilitate high sensitivity detection for quantitation of low-level impurities; and because selectivity can readily be controlled via mobile phase optimization. Here proper wavelength selection is a critical part of method development. When choosing a detection wavelength, the following factors need to be taken into consideration:
1. The major components must have suitable dynamic range at the selected wavelength.
2. All impurities should be detected with suitable sensitivity at the selected wavelength.
3. Mobile phase should not show strong background absorption at the selected wavelength.
4. Detectability should be maximized for components of interest and minimized for those of blank interference of mobile phase.

The chromatographic method should be tested to ensure that no impurities or degradants co-elute with the main peak of interest. Homogeneity of the main peak can often be determined by either direct or indirect evaluation of its peak purity. Direct evaluation can be performed in-line by employing photo-diode array (PDA) detection, LC-MS. However, PDA and LC-MS evaluation will not work if the degradants have similar UV profiles and the same molecular weight, such as diastereomers. Indirect evaluation of peak purity can be accomplished by changing one or more chromatographic parameters (column, mobile phase, gradient composition, etc.) that will significantly impact the separation selectivity. The resulting impurity profile is then compared against that of the original method. If the number of degradant peaks is the same in both separations, and if the area percent of the main component is the same in both separations, then all the degradants have probably been resolved. Mass balance deficits can be investigated in a number of ways:

1) Use gradient HPLC to elute compounds over a wide polarity range,
2) Compare UV profiles because imbalance may occur from different UV responses;

PDA can be used to detect peaks with different absorption wavelengths,
3) Check for highly retained compounds by using a stronger mobile phase or by using TLC to monitor for sample components that have not migrated from the point of origin.

4) Evaluate void-volume peaks for the presence of poorly retained degradants.

5) Look for undetected peaks from non-chromophoric degradants by alternative detection such as LC/MS, GC, RI, chemiluminescence nitrogen detector, TLC (with I₂ or acid/charring visualization).

6) Account for volatile degradants by GC.

7) Monitor for oligomers/polymers by size exclusion (SEC) or gel permeation chromatography (GPC).

1.4 Method Validation

Method validation is the process used to demonstrate that an analytical method is suitable for its intended purpose. For pharmaceutical methods, guidelines from the United States Pharmacopoeia (USP)⁶⁹, International Conference on Harmonization (ICH)⁷⁰, Food and Drug Administration (FDA) and several literatures⁷¹-⁷³ provide a framework for performing such validations. In general, methods for routine analysis, standardization or regulatory submission must include studies on specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness.⁶⁹-⁷⁷ Although there is general agreement about what type of studies should be done, there is great diversity in how they are performed. The literature contains diverse approaches to performing validations. This approach should be viewed with the understanding that validation requirements are continually changing and vary widely, depending on the type of drug being tested, the stage of drug development, and the regulatory group that will review the drug application.
The process of validating a method cannot be separated from the actual development of the method conditions, because the developer will not know whether the method conditions are acceptable until validation studies are performed. The development and validation of a new analytical method may therefore be an iterative process. Results of validation studies may indicate that a change in the procedure is necessary, which may then require revalidation. During each validation study, key method parameters are determined and then used for all subsequent validation steps. To minimize repetitious studies and ensure that the validation data are generated under conditions equivalent to the final procedure, we recommend the following sequence of studies.

Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development. Analytical validation is the cornerstone of process validation. Without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new developed methods are validated.

1.5 Literature Study

In literature shows several chromatographic novel or modified techniques (such as HPLC and GC) were reported for assay and related substances determination in different-different drug substance (API) and/or drug product. Literature study revealed that RP-LC methods have been developed and validated for the detection and quantification of drug substance and its related impurities. Miseljic et al, in 2008, has been developed a gradient RP-LC method for the detection and quantification of norfloxacin and its major
impurities in norfloxacin-containing pharmaceuticals. S.R. Chitturi et al also report’s the development and validation of gradient RP-LC method for the determination of process and degradation related substances in lopinavir bulk drugs. For the determination of azithromycin impurities in tablets as pharmaceutical form LC method with UV detection has been developed by L. Miguel et al. A selective gradient LC method for the determination of oxytocin (OT) and its related substances in bulk drugs has been developed by D. Ashena et al. The isocratic LC method has been developed by H. K. Chepkwony et al for the analysis of bulk samples of roxithromycin and its related substance determination. Capillary zone electrophoresis method for the separation and determination of tiapride hydrochloride and its two related impurities in pharmaceutical formulations has also developed and validated by Y. Wang et al. Stress degradation behavior of entacapone was studied by S. K. Shetty et al and accordingly, stability indicating gradient RP-LC related substances and assay method has been developed for the quantitative determination of entacapone in bulk drugs. Several literature methods reported for the development and validation of RP-LC method for simultaneous separation and determination of API (e.g. paracetamol, nimesulide, donepezil hydrochloride and atazanavir sulfate) and its related compounds in bulk drugs and/or pharmaceutical formulations. M. Vuletic et al (2005) report’s the detection of unknown impurities in simvastatin substance and tablets at a 0.2% level by LC-UV/PDA technique and those were identified by liquid chromatography/tandem mass spectrometry. A. Garg et al studied the forced degradation of fentanyl and identification and analysis of impurities and degradants.
However, the continuous development in the area of synthesis of drug substance (API) and drug product created a strong scientific and commercial desire for the development of new, sensitive and accurate chromatographic methods for analysis of drug substances and/or drug products. Furthermore the development of stability indicating chromatographic methods for impurity profile study of API is of a great practical and commercial interest. Hence for the research study we choose those API’s whose analytical methods were not reported anywhere and develop a new or novel analytical method those selected API’s.

1.6 Objective and Scope:

The goal of the presented work is to develop and validate the chromatographic methods (HPLC and GC) for determination of process as well as degradation related impurities in API’s. The research work provides the information about GC and LC techniques and its application in pharmaceutical industry. It also provides the valuable information about the product stability. In research work the forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, better understanding of the potential liabilities of the drug molecule chemistry, and the resolution of stability related problems.

APIs selected for the present study are as follows:

1. Quetiapine Hemifumarate
2. Paliperidone
3. Bosentan Monohydrate
4. Cinacalcet Hydrochloride
5. Memantine Hydrochloride
6. Oxantel Pamoate

This Ph.D. work was undertaken as a part of a comprehensive research program in the group for development of suitable chromatographic methods for API, Impurity profile study of API, and development of stability indicating methods for API with the following objectives: 1) Literature survey on analytical method available for selected API’s, 2) Selection of chromatographic techniques (HPLC or GC), 3) Forced degradation studies, 4) Preliminary separation studies on stressed sample, 5) Optimization of LC conditions using stressed sample, 6) Peak purity check using PDA detector (When LC system used), 7) Peak homogeneity confirmed by GC-MS (When impurity profiling studied by GC), 8) Finalizing the HPLC or GC method after proper separation of impurities and degradation products, 9) Validation of developed analytical method as per ICH guideline.

All the methods described in the present work are simple, rapid, reliable and validated. The developed analytical methods reported in the present research work are significantly improved and advanced from those reported in the literature or mentioned in different pharmacopoeias for respective API. The methods could be used not only for quality control but also for pharmaceutical formulations. Validated stability-indicating HPLC and/or GC methods for impurity profiling study of Quetiapine hemifumarate, Paliperidone, Bosentan monohydrate, Cinacalcet hydrochloride, Memantine hydrochloride and Oxantel pamoate have been developed and described in Chapter-2, 3, 4, 5, 6 and 7 respectively. Moreover this work is well supported by structural characterization of newer
molecules by various techniques such as FTIR, $^1$H-NMR, and LC-MS. Hence with the help of above scope present work is summarized as chapter wise as follows:

1) Chapter-2 covers the development and validation of stability indicating HPLC and GC method for the determination of potential impurities in Quetiapine Hemifumarate API. In the manufacturing process of quetiapine hemifumarate, impurities were generated as chromophoric as well as non-chromophoric in nature. Hence in this chapter, impurity profile of quetiapine hemifumarate was studied by two different chromatographic technique viz., HPLC and GC. The validated stability indicating HPLC method was developed for the determination of chromophoric impurities in quetiapine. Also validated GC method was developed for the determination of non-chromophoric impurities in quetiapine and covered in same chapter.

2) Chapter-3 covers the development and validation of stability indicating HPLC method for the determination of potential impurities in Paliperidone API. In the manufacturing process of paliperidone, five impurities related to process and degradation were generated and details of the same are presented.

3) Chapter-4 covers the development and validation of stability indicating HPLC method for the determination of potential impurities in bosentan monohydrate API. The detailed impurity profiling study has been presented.

4) Chapter-5 covers the development and validation of stability indicating HPLC method for the determination of potential impurities in Cinacalcet hydrochloride API. The developed method is capable to separate the thirteen process related impurities.

5) Chapter-6 covers the development and validation of stability indicating GC method for the determination of potential non-chromophoric impurities in Memantaine hydrochloride
because memantine and its impurities are UV inactive. The stability indicating property of developed method was confirmed by GC-MS analysis.

3) Chapter-7 covers the development and validation of stability indicating HPLC method for the determination of potential impurities in Oxantel pamoate API.
References


5. European Medicines Agency (EMA), ICH Topic Q3C (R4), Impurities: Guideline for Residual Solvents, (2009), CPMP/ICH/283/95


69. The United States Pharmacopeia, Validation of Compendial Methods, 32nd Edn. (USP 32), Section <1225> (2009).


