CHAPTER-I

Introduction
1.1 PHARMACEUTICAL INDUSTRY

Pharmaceutical word originates from the Greek word “pharmakeutikos” (Oxford Dictionary, 2000). The Pharmaceutical industry cultivates, manufactures the drugs as medicines (McGuire, 2007). German dye producers successfully documented basic technology in organic separation (Top Pharma Establishments, 1930).

The growth of artificial organic technologies permitted scientists to methodically differ the arrangement of chemicals and evolution in the developing science of pharmacology lengthened their capability to assess the biotic properties of these arrangement alterations (Walter Sneader, 2005).

During initial 19th century, Hermann Emil Fischer revealed their detection of diethylbarbituic acid, made from diethylmalonic acid, phosphorus oxychloride and urea. This helps to prompts sleeping tendency in animals mainly in dogs and this detection was propriety and authorized to Bayer Pharmaceuticals, sold the material with the brand name Veronal as a sleeping assistance in the start of 1904 (Rasmussen Nicolas, 2006).

Later organized research of the outcome of arrangement modifications on effectiveness and extent of act directed to the unearthing of Phenobarbital (Yasiry, 2012) by Bayer in 1911-12; the anti-epileptic action was founded. Phenobarbital was amongst the maximum broadly recommended drug for the management of epilepsy (LF Munoz, 2005, Drug Abuse Control Amendments, 1965,) over the year 1970) (Sedative Hypnotic Drugs, 1956) and till now placed in the vital medications list of WHO (Rosenfeld L, 2002).The 1950-60 saw augmented consciousness of the addictive nature which leads growing limitations on usage and increasing regulatory lapse of prescribers (Zaffiri L, 2012).

Oncology drugs were included for research during 1970; from the year 1978, India grabbed as the key center of pharmaceutical manufacturing without patent regimen
Subsequently after 2008, pharmaceutical organizations consistently increasing the price of trademark prescribed drugs to balance the deteriorating incomes due to tremendous increase of generics in the market (Abraham EP, 1987).

1.2 RESEARCH & DEVELOPMENT

Drug discovery plays a vital role in identification and designing new drugs. Historically more number of medicines produced either by separating the active moiety from old-fashioned cures or by serendipitous discovery (Kingston, 2004). Recent emphases is on accepting the metabolic ways linked to an illness form or pathogen and influencing these conduits by means of molecular biology or biochemistry and new drug research activities has conventionally agreed out by education and research institutions (Nelson ML, 2011).

Main aim of drug progress is to define suitable formulation preparation and medicating mode and also to founded the safety of drug. Investigation in these fields usually comprises a grouping of in vitro and in vivo readings and followed by clinical trials. The sum of investment needed for late phase progress has made it as strong point of the bigger pharmaceutical establishments. (Wright JM, 2009).

Regularly, huge multinational companies display upright mixing, contributing in a comprehensive variety of new drug identification, development, production, quality control, marketing, sales, and dispersal of new approved drugs (Stason WB, 1996). Lesser scale companies on the other side were focus on an exact aspect like identifying drug candidates or developing dosages for administer (Black JW, 1964) and later joint agreements among study organizations and big pharma companies were made to discover the activity of new drug compounds (LvPerkovic, 2012). Now a day, global pharma companies were moved towards engaging on contract research organizations to accomplish drug development programs. (Ray Moynihan, 2005).
In the United states, novel pharmaceutical drugs must be permitted by the Food and Drug Administration (FDA) on safe and efficacy of drugs (Perry, 2012). This procedure usually contains filling of an Investigational New drug application with adequate supportive pre-clinical facts to backing the scheduled of human trials. After post approval of IND application (Bhandari M, 2007), submission of the new drug application will be accepted for the review of supportive and post evaluation of data. The products has shown a constructive benefit-risk assessment, authorization to establishments was released to sell the product in market (Moynihan R, 2007). FDA updates the withdrawal as well as on approved drugs in Orange Book (Liberti L, 2011).

In the year of 2011, worldwide production of drugs was around 954 USD billion; the development was slowed down fairly in Europe and North America [Herper, 2011]. Emerging markets like China, Russia, South Korea and Mexico outperformed and increasing at the pace of 81 percent per annum (Hyder, 2010).

1.3 PHARMACEUTICAL DRUGS
Pharmaceutical drugs according to Steven Mpaul are “a chemical ingredient or sole active component or a blend of added pharmacologically active material”. (Steven Mpaul, 2010). Drugs were classified by numerous ways (Banta DH ,2001) and Pharmaceuticals are differentiated in numerous additional collections on their source like manner of deed and their pharmacological deed or commotion, way of administration, biological scheme impacted, or therapeutic impacts (Barton JH ,2005).

1.4 IMPURITIES
and Health products; (http/www.mccza.com/Guidelines/Human medicine) are stressing on the pureness, safety and the identification of impurities in drug substance and products. Quality of a Pharmaceutical product and substances is to limit the content of impurities, as their existence, even in small quantity, may disturb the drug safety and efficiency.

ICH guidelines (ICH, Q6A, 2006) were aimed with the joint exertions of regulators and trade representative from the European Union, Japan and the United States. As per ICH (Q1A (R2), 2006), novel drug ingredients are articulated from two viewing platform:

1. Chemistry characteristics comprise cataloging and identification of impurities, collection of impurities in specifications, and an ephemeral conversation of diagnostic procedures; and
2. Safety features comprise of definite regulation for qualifying those impurities that did not exist or existed at significantly lesser amount, in consignments of a novel drug ingredient used in safety and clinical readings.

As per ICH guidelines, (ICH Q3A (R2), 2006; Q3B (R2), 2006), unknown impurities connected with bulk drug and dosage form, higher than the quantification threshold should be notorious.

Table 1.1 Acceptance criteria for impurities

<table>
<thead>
<tr>
<th>Maximum Daily Dose¹</th>
<th>Reporting Threshold²,³</th>
<th>Identification Threshold³</th>
<th>Quantification Threshold³</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 gram per day</td>
<td>0.05%</td>
<td>0.10% or 1.0 mg/day intake (whichever is lower)</td>
<td>0.15% or 1.0 mg/day intake (whichever is lower)</td>
</tr>
<tr>
<td>&gt; 2 gram per day</td>
<td>0.03%</td>
<td>0.05%</td>
<td>0.05%</td>
</tr>
</tbody>
</table>
1. The quantity of drug material administered per day.
2. Higher reporting threshold should be scientifically vindicated.
3. Lower thresholds can be suitable if the impurity is oddly toxic

1.4.1 Types of Impurities [(ICH Q3A (R2), 2006) and (Q3B (R2), 2006)]
The main culprit in the quality and safety of drugs are the impurities. The author has tried to classify them with the help of below Chart (Figure 1.1).

![Figure 1.1: Flow Diagram of Different types of Impurities](image-url)
1.4.1.1 Process impurity
These are the impurities either getting formed / generated during the course of manufacturing of the drug. These impurities origin needs to be investigated and controlled within the specified limit. The synthetic process should support the non-generation of process related impurities and controlled up to maximum extent.

1.4.1.2 Degradant Impurities
This type of impurity will get generated while product under go degradation which generate new impurities or else degradation may leads to formation of byproduct on storage of drugs under various atmospheric conditions.

1.4.1.3 Impurities due to Contamination
The main source is cross contamination while handling and manufacturing. Contamination can happen at dissimilar steps of the making; other sources like from input raw material, reagents, reactors, equipment’s, instruments, packaging material and many other external sources.

1.4.1.4 Polymorphic Impurities
Polymorphism is the ability of the organic compound to crystallize as more than one different crystal species and polymorphs are different crystalline forms of the same pure compound. The final product should be the required form.

1.4.1.5 Enantiomeric & Stereochemical Impurities
Few chemical compounds having tendency to be optical active which leads these compounds have the ability to rotate the plane of polarized light. Drugs having optically active nature owe to formation of unwanted isomer during synthesis and these are called optical impurities which need to be controlled within specified limit.

1.4.1.6 Carry over impurities
The possible source for impurities is mainly during the synthesis of drug and might be unreacted input raw materials, intermediates, reagents, byproducts, catalyst and other sources.
Different Regions has various set of Regulations on control of impurities. ICH Guidelines was most recognized and universally accepted. The history of Regulation on Impurity was articulated below.

**History of major Regulations in the field of impurities**

1996: The ICH impurity guideline ICH Q3A “Impurities in New Drug Substances”
   (“Identification should be at a level lower than 0.1 percent”.)

2002: ICH Q3A (R1) Guideline “Impurities in New Drug Substances”
   (To consider for potential GTIs by stating: “lesser thresholds may be appropriate for unusually toxic impurities”).

2004: the European Medicines Agency (EMEA) issued their “Guideline on the limits of Genotoxic Impurities”

2008: US FDA draft guidance for industry entitled: “Genotoxic and Carcinogenic Impurities – Recommended”

2009: Paper on ICH Q7 Guideline

2013: ICH Q7 Guideline (ICH, Q7, 2006)

Source: ICH Guidelines.
The Flow chart represents (Figure 1.2) the various steps involved to characterize the impurities in a Drug.

Figure 1.2: Flow chart of Impurity Characterization

1.4.2 Genotoxic Impurity

Genotoxicity is the broad term to indicate for any toxic changes in the genetic substance irrespective of the tool by which the variation is encouraged.

Genotoxic impurities have also been well-defined as an “impurity that has been established to be genotoxic in a suitable Genotoxicity assessment exemplary, e.g., bacterial gene mutation (Ames) test”. A probable genotoxic impurity (PGI) has been defined as an “Impurity that displays (a) structural attentive or alert (s) for genotoxicity but not been verified in an investigational trial model and theoretically transmits to genotoxicity, not to extent of existence or nonexistence impurity” (EMEA, 2012).

ICH has published a guideline on the determination and the control of DNA reactive (mutagenic) impurities in medicinal goods. The guideline is enabled: "M7: Assessment (Figure 1.3) and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk". Higher levels is adequate for convinced conditions like life-threatening conditions, life expectancy less than five years, human disclosure much better from further foundations. High effectiveness complexes like N-nitroso necessity to be restricted lower generic TTC. This advice doesn't put on to biological or biotechnologically factory-made medicinal, oligonucleotides, herbals, fermented, peptides and crude materials of animal or plant source.

To evaluate the safety of genotoxic potential impurities more background information need to be collected by the way of reviewing all accessible evidence from Safety Data Sheets, publications evaluation, Evaluation of structures, regulatory and government activity records (TOXNET at National Library of Medicine web site, CalEPA, National Toxicology Program, Cancer Potency Database from UC Berkeley. Few sophisticated soft wares are available such as Derek, Lead Scope, and Multicase for Genotoxic impurity identification and followed by Ames testing to get precise results; by utilizing all the information which helps in setting the limits for the impurities. (L Müller, 2006, RS Satoskar, 2001).
1.4.2.1 Classifications of Genotoxic Impurities

Genotoxic Impurities are classified into five classes

Class -1
Impurities identified to be genotoxic (mutagenic) and carcinogenic known over animal carcinogens with dependable facts for a genotoxic mechanism and human carcinogens. The genotoxic environment of the impurity is established by means of distributed data on the chemical structure.
Class -2
Impurities identified to be genotoxic (mutagenic), but with unidentified carcinogenic probable. This collection comprises of impurities with established mutagenicity created on challenging of the impurity in conservative genotoxicity tests.

Class -3
Impurities that have a warning construction and very distinct to the structure of the active substance, unidentified genotoxic (mutagenic) potential and this assembly contains impurities with useful moieties which connected to genotoxicity founded on structure. However, these moieties not tested as isolated materials and these substances are recognized based on chemistry and also utilizing knowledge based proficient classifications for structure action associations.

Class -4
Impurities with an alerting structure related to the active substance and impurities that consists of alerting functional moiety that is united with the structure of the active substance.

Class -5
Absence of functional groups alert or any indication on potential genotoxic impurity.

According to Center for Drug and Evaluation Research, the descriptive flow diagram for Impurity identification was given in below Decision Tree flow Diagram (Figure 1.4) (Department of health and human science, CDER, 2008)
Figure 1.4: Decision Tree flow Diagram of Impurity
The genotoxic nature of compounds were determined based on functional groups and below representative diagram (Figure 1.5) represents structural alerts.

**Structural Alerts – Pharmaceutical Impurities** (L. Müller, 2006)

**Group 1: Aromatic Groups**

- N-Hydroxyaryl
- N-Acylated anilinoaryl
- Az-aril N-oxides
- Aminonitrosamines and alkylated anilinoaryl

**Group 2: Alkyl and Aryl Groups**

- Aldehydes
- N-Methyliso
- N-Nitrosamines
- Nitro Compounds
- Carbonates (Uremones)
- Epoxides
- Aziridines
- Propliolactones
- Propiolactones
- S or N Mustards (beta haloethyl)
- Hydrazines and Azo Compounds

**Group 3: Heteroatomic Groups**

- Michael-reactive Acceptors
- Alkyl Ethers of Phosphonates or Sulfonates
- Halo-alkenes
- Primary Halides (Alkyl and aryl-CH3)

**Figure 1.5: Potential Genotoxic Functional Structures**
1.5 Quality Control of Drugs

Quality (PD Sethi, 1997)] is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods, there can be no “second quality” in drugs. Quality control is an idea, which endeavors to yield a perfect produce by sequence of procedures intended to stop and remove faults at various stages of manufacturing. Pharmaceutical analysis is the branch of science which deals with identification of substances and determination of amount present in particular sample. Pharmaceutical analysis covers the bulk materials, dosage forms and more recently, biological samples in support of bio-pharmaceutical and pharmacokinetic studies.

1.5.1. Definition:

Pharmaceutical analysis is well defined as “a procedure or a sequence of procedures to identify and/or measure a material or drug, the components of a pharmaceutical solution or combination or the identification of the assemblies of chemical substances needed for formulating the product.”

1.5.2. Classification:

Pharmaceutical analysis broadly categorized into two groups (Talanta, 1989).

1.5.2.1. Qualitative Analysis:

The method of determination of substances or constituents exist in an unknown compound is called qualitative research.

1.5.2.2. Reckonable Analysis:

In a Compound, the exact contents of various components to be determined like Ca, C and O component in calcium carbonate, such type of analysis will be called as Quantitative estimation analysis. This analysis is divided into following type

- Physical analysis
- Chemical: qualitative and quantitative analysis.
- Physico-chemical analysis
- Microbiological analysis.
1.5.3. **Steps involved in Quantitative analysis** (Skoog DA, 1988)

- Selection of method of analysis.
- Sampling.
- Preparation of sample Solution.
- Elimination of interferences.
- Calibration and measurement.
- Calculation of results.
- Evaluating results and their reliability.
- Factors affecting the choice of analytical methods.
- Type of analysis required.
- Problem arising from the nature of the material.
- Possible interference from components of the material other than those of interest.
- Concentration range, which needs to be investigated.
- Accuracy required.
- Facilities available.
- Time required for complete analysis.

1.6 CHROMATOGRAPHY

The Liquid term ‘Chromatography’ coined to include the techniques intended at the separation of mixture on the characteristic of distribution properties in between an inactive and a liquid phase.

Chromatography analyses are related to the separation of substances by affinity difference between the mobile phase and stationary phase. The mobile phase is typically a polar liquid or a gas and the stationary phase is generally a compact or might having immobilized liquid and comparative affinity related to soluble characteristic, adsorption, size or polar charge.

There are numerous types of chromatography, nevertheless all include the dissolving the compound which need to analyses with a polar or non polar liquid called as mobile phase and the passage of this liquid over a stationary phase, often a solid or
liquid-coated compact. The mobile phase comes into interaction with the stationary phase, the analyte substances gets dissolved or adsorbed by solid phase and adsorption based on affinity with solid phase and the higher amount of analyte getting retained indicates the sluggish on the progress of analysis over chromatographic apparatus and dissimilar materials travel through solid at various rates, preferably ensuing in noticeably recognizable retention times for each and all materials.

During the analysis by thin-layer chromatography, solid phase in the form of plates were used and result patterns are regularly observed under ultraviolet light radiation, or else by staining method using chemicals like iodine kept in glass compartment or potassium permanganate. In Gas chromatographic method, the separation of materials and detection based on variation in the ionization levels of a flame at the output end of the column or may be variations in the electrical conductivity properties of the gas mixture at outlet end of the column.

1.6.1 High Performance Liquid Chromatography

HPLC was resulting from classical column chromatography (RE Shoup,1986) and has found an important place in analytical techniques. It is a physical separation technique in which a sample dissolved in a liquid is injected into a column packed with small particles and it is separated into its constituent components. It is probably the most important and widely used analytical technique for quantitative analysis of organics and bimolecules, also applicable to much kind of samples. Liquid chromatography employs a fluid mobile phase to separate the constituents of a blend. The stationary phase either in the form of liquid or a solid and these mixtures were liquefied in a solvent and allowed to flow over a column beneath a high pressure. The separation of the components in a mixture takes place depending upon the degree of interface among the components of solute, stationary phase and the inactive phase.

The interface of the solute with mobile and stationary phases was altered by adopting various solvents and stationary packing materials. Due to these facts and also capability of separating widespread variety of various natures of chemical mixtures,
High performance liquid chromatography attains a high degree of adaptability which never found in other chromatographic systems.

1.6.1.1 Types of HPLC

1.6.1.1.1 Normal phase HPLC:
The principle of separation is same as that of thin layer chromatography or column chromatography and even though it is mentioned as "normal", it is not an indication it is regularly used form of HPLC. The normal terminology indicates the stationary phase or tubular columns are polar in nature and solvent liquid mobile phase might bear non-polar characteristic. (Horvath C, 1967)

1.6.1.1.2 Reverse phase HPLC:
In this case, the stationary phase usually made up of inert silica and outer layer altered in the way to become nonpolar in nature and it was made by attaching various elongated hydrocarbon chains characteristically with either containing 8 or 18 carbon atoms hydrocarbon chains. Reversed phase Liquid chromatography is the furthermore frequently used form of High performance Liquid chromatography and during analysis, compounds having polar in nature will move quickly throughout the solid stationary phase.

1.6.1.2 Practical Description of the HPLC Parts
- Mobile phase reservoir
- Filtering aid
- Dual mode Pumps
- Auto sampler injector
- Columns
- Detector
- Data processing module
1.6.1.2.1 Mobile phase reservoir
The solvent reservoir is in the form of bottle which was either made by glass or by plastic. The reservoir bottle is with screwed and threaded colored closures, and all the tubes used for carrying the mobile phase was made from Teflon.

1.6.1.2.2 Pump
High pressure pumps were used to pump the liquid phase over packed solid stationary phases and stationary phase packing having defined particle size and pressure needed to pump liquid versus size of the particle were direct relational. Rate of flow of liquid phase consistency was another critical feature of pumps and extent of precise in controlling the flow correlates with cost of pump and performance of pumps in a straight line affects the elution time and pattern as well as in reproducibility of analysis results. Between the numerous solvent delivery structures (direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump consists of either twin or triple pistons is commonly utilized in chromatography because this system provides minimal baseline fluctuation with consistency in the rate of flow of liquids. HPLC pumps might bear the following important features:

- Range of Flow from 0.01 to 5mL/minute
- Flow rate Consistency: not beyond 1%
- Maximum pressure: 300MPa.
- Inbuilt degassing system with membrane filtering aid.

A separation in which the mobile phase composition remains constant ratio throughout the analysis procedure is termed isocratic (meaning constant composition). The word was coined by Csaba Horvath who was one of the pioneers of HPLC and separation in which the mobile phase composition is changing gradually with timings during the separation process is described as a gradient elution.

1.6.1.2.3 Injector
Sample introduction into the chromatographic system can be managed by numerous methods and of the unassuming technique by using an injection valve. In modern days and new refined Liquid chromatographic systems, programmed sampling devices are
combined in which the sample is introduced to the chromatographic systems with the assistance of auto samplers and programmed with microprocessors chips. The instrument factors like rate of flow, ramping elution timings, capacity to be injected, etc. are preferred, programmed in memory and chronologically effected on repeated injections.

1.6.1.2.4 Column
In High performance Liquid chromatography, two types of columns are available. Normal phase and reversed phase columns. The compounds having moderate polar or non polar characteristic nature reflect exceptional separation in normal phase columns. Initially, the separation of compounds mixtures effected gradually by variance adsorption on a stationary silica phase, but now it seems that partition coefficient plays a major role while interacting with the compounds of polar silanol groups embedded on the silica (Lloyd R Snyder, 2006).

Column Efficiency
Column effectiveness usually denotes the performance of the stationary phase to meet separation goal. Efficiency of a column can be monitored by numerous ways like tailing factor or appearance of a front. During the course of analysis it is very vital, since most chromatographic peaks may not fall under the purview of Gaussian distribution which having calculation and limits for preferred peak shape.

Column-packing materials
The heart of the HPLC analysis is column, silica were commonly and frequently utilized material to manufacture the packing material to fill the tubular column and it may contains a polymer of siloxane linkages (Si-O-Si) in a unbending three dimensional building comprising entomb linking apertures with a varied range of surface areas extending from 100 to 800 m²/g with size of the particles varies from 3 to 50µ. (Lloyd R Snyder, 2006).

The different types of stationary phases are available with various phase Description like Si-Silica, C1-Tri methyl silane, C2-RP-2, Dimethyl, C3-Propyl, C4-Butyl, C6-
Hexyl, C8-RP-8, LC8, Octyl, C18-ODS, RP-18, LC18, Octadecyl, Phenyl APS, Amino, Amino propyl silyl, NO₂-NitroSi NO₂, SAXSB, Quaternary amine, Strong BaseSi CH₂CH₂CH₂N + (CH₃)₃, SCX (SA, Sulfonic acid, Strong Acid), WAX (PEI, DEAE, Polyethyleneimine), WCX (CM, Carboxymethyl, Weak Acid Si CH₂COOH), Cyano (CPS, PCN, Cyano, Cyanopropyl, NitrileSi CH₂CH₂CH₂CN). (Lloyd R Snyder, 2006)

### 1.6.1.2.5 Detector

Detectors were categorized into two types as bulk and solute property detectors. Bulk property detectors operate by the way of observing property of the liquid like refractive index, conductance or dielectric constant. Bulk property detectors have a feature of emitting limited signal during nonexistence of a solute and which limits the boundaries on vast usage of these type detectors. Tallying of a small concentration of analyte would able to sum up only a minor augmentation on inducing electric signal background signal of mobile phase. Bulk detectors largely extract lower capacity of sensitivity and not appropriate for trace analysis and gradient elution. This is of tendency to generate signals to mobile phase and in gradient the change in mobile phase ratio leads to change in signal. A Solute property detector reacts to particular property of analyte and detectors falls under this category are ultraviolet absorbance, fluorescence detector and electrochemical detectors. These types of detectors have high sensitive of detection at lower levels.

- UV-Visible absorbance detector
- Refractive Index detector (RI)
- Photo-diode array detector (PDA)
- Fluorescence detector
- Evaporative light scattering detector (ELSD)
- Electro chemical detector (ECD)
- Conductivity Detector
- Mass detector (MS)
- FTIR
- Light scattering detector (LSD)
The two most commonly used HPLC detectors are UV absorbance and RI detector.

**Ultraviolet-Visible Absorbance Detectors**

Ultra violet visible detectors were amongst the principal mode of sensors used for chromatography analysis. It remain widespread prevailed for chromatographic analysis are permanent wavelength detectors which function from a distinct light cradle, variable wavelength detectors which uses a band source and an monochromator, third one is quick scanning detectors which are commonly built on a linear photodiode array detector.

**Refractive Index Detectors**

Refractive Index detectors popularly called as universal detectors. This detector reacts to variations in the refractive index of the mobile phase and deviations of signals is due to solute concentration, temperature, pressure, dissolved gases, and changes in mobile phase ratios. This detector only for isocratic separations and need to be functioned at extreme sensitivity with controlled temperature and mobile phase. These detectors were useful for separation of polymer or macromolecular separations.

1.7 METHOD DEVELOPMENT

The development of any new or improved method for the analysis of analyte usually depends on tailoring the existing analytical approaches and instrumentation. Method development (Lloyd R Snyder, 2006) usually involves selecting the method requirements and type of instrumentation. In the development stage of HPLC method, decision regarding the choice of column, mobile phase, detector and method of quantitation must be addressed.

Once the instrumentation has been selected, it is important to determine the chromatographic parameters for the analyte of interest. The characteristic of the analyte(s) is very useful to select the nature of the column to be used, establish the approximate configuration and pH of the mobile phase for separation of the components, wavelength to be employed for detection of the component. Such information might be available in the literature for the analyte or related...
compounds. This is followed by optimization and preliminary evaluation of the method.

Optimization criteria must be determined with cognizance of the goals of any new method. Initial analytical parameters of merit like sensitivity (measured as response per amount injected), limit of detection, limit of quantitation and linearity of calibration plots are to be determined. As a precautionary measure, it is important that method development be performed using only the analytical standards that are highly pure and have been well identified and characterized and whose purity is known.

**Systematic approach for chromatographic separation of pharmaceutical compounds** (Lloyd R Snyder, 2006, Xiang Y, 2006, R pauls, 1988)

The principal step in the method development is to characterize the drug whether it is regular or special. The regular compounds are those that are neutral or ionic. The inorganic ions, bio-molecules, carbohydrates, isomers, enantiomers and synthetic polymers, etc are called special compounds. The selection of initial conditions for regular compounds depends on the sample type.

- Literature search of the sample
- Properties of sample like its molecular structure, molecular formula, solubility, pH, pKa value or any other characteristics.
- Purpose of separation.
- Proper sampling technique.
- Selection of Detectors.
- Selection of Column and Flow rate
- Selection of mobile phase.
- Optimisation of method with respect to temperature, sample size, mobile phase saturation
- Verification of method
- Method validation.
1.9. METHOD VALIDATION

Method validation is the procedure of defining an analytical prerequisite, and approving that the method under consideration has performance abilities constant with what the application needs. As per ICH “Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use”.

Validation is necessary if:

- New method developed for specific delinquent
- Established method was brushed up to include enhancements or prolonged to a new problem
- Quality control lab shows a recognized method is varying with time.
- To establish the equivalency between two methods, e.g. a new method and a standard.

Furthermore revalidation may be essential in the subsequent conditions:

- Modification in the process adopted to produce the drug substances
- Alteration in the configuration or mixture ratio of components in finished product.
- Any modification in the analytical procedure and parameters.

The Following types of tests need to be validated

Identification tests:
Identification tests are proposed to confirm the individuality of an analyte in a mixture of sample. This is normally achieved by evaluation of a nature and characteristic of the sample with that of standard samples.

Quantitative tests or limit tests for control of impurities:
Analysis for impurities can be quantitative test and a limit test for the impurity content. Both the test are proposed to reproduce the purity characteristics of the compound and different validation parameters and features are essential for a quantitative test rather than for a limit test.

Quantitative tests of the active Substances:
Assay testing methods are established to measure and quantify the content of active substance existing in a given compound. The assay signifies a quantitative measurement of the main constituent(s) in the finished drugs and also for the drug product or formulated product. The Validation characteristics requirements also applicable while assaying for the active drugs or other selected component in drug products.

The analytical methods need to be validated and checked for following parameters as per ICH Guidelines:

- System suitability Parameter test
- Specificity or Forced degradation test
- System Precision Parameter
- Repeatability or Method Precision
- Intermediate Precision
- Linearity
- Range
- Accuracy or Recovery
- Limit of Detection
- Limit of Quantification
- Robustness
1.9 OBJECTIVE OF RESEARCH WORK

Safety and efficacy are the two most important aspects to evaluate in any drug substances and drug product. Hence it is crucial to maintain good quality of drugs which are used to treat different diseases. At the same time it requires need for development of newer, efficient and economical analytical methods for estimation of impurities in various drugs for improving the product quality and manufacturing efficiency of the drug product. Hence, an attempt has been made to identify and quantify the impurities, potential genotoxic impurities, degradation and analysis of the drug by HPLC method. The Results of the Investigation are presented in the Thesis.

The Present research work focused on the Development of New RP-HPLC methods for the Quantification of the impurities/Genotoxic impurities of widely used selective lifesaving Drugs. The Work also includes the Validation of the proposed method according to ICH guidelines. The List of the Drugs and their profile selected are presented in the Table 1.2 and Structures are presented in Table 1.3.
Table 1.2: Profiles of the Drugs Chosen

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug Name/ CAS No</th>
<th>Therapeutic Category</th>
<th>IUPAC Name</th>
<th>Molecular formulae and mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milnacipran HCl</td>
<td>Fibromyalgia</td>
<td>“(±)-[1R(S),2S(R)]-2-(aminomethyl)-N,N-diethyl-11-phenylcyclopropane carboxamide hydrochloride”</td>
<td>C_{13}H_{22}N_{2}O.HCl 282.8</td>
</tr>
<tr>
<td>2</td>
<td>Lurasidone HCl</td>
<td>Depressive disorder and Schizophrenia</td>
<td>“3aR,4S,7R,7aS)-2-(((1R,2R)-2-[[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]methyl]Cyclohexyl methyl] hexahydro-1H-4,7-methanisoindol-1,3-dione HCl”</td>
<td>C_{28}H_{36}N_{4}O_{2}S.HCl 528.87</td>
</tr>
<tr>
<td>3</td>
<td>Dabigatran etexilate mesylate 92623-85-3</td>
<td>Thrombin Inhibitor</td>
<td>“β-Alanine,N-[[2-[[4[[[(hexyl oxy)carbonyl]amino]iminomethyl]phenyl]amino]methyl]-1-methyl-1H-benzimidazol-5yl carbonyl]-N-2-pyridinylethyl ester”</td>
<td>C_{34}H_{41}N_{7}O_{5} • CH_{4}O_{3}S 723.86</td>
</tr>
<tr>
<td>4</td>
<td>Ropinirole HCl</td>
<td>Anti-Parkinson disease</td>
<td>“4-[2-(N, N-Di-npropylamino)ethyl]-2(3H)-indolone, HCl”</td>
<td>C_{15}H_{24}N_{2}O.HCl 296.84</td>
</tr>
<tr>
<td>5</td>
<td>Irbesartan 114772-54-2</td>
<td>Anti-hypertensive</td>
<td>“2-buty1-3-[p-(o-1Htetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro [4.4]non-1-en 4-one”</td>
<td>C_{14}H_{10}BrN 272.14</td>
</tr>
<tr>
<td>6</td>
<td>Montelukast Sodium 158966-92-8</td>
<td>Anti-Asthmatic</td>
<td>“([R-(E)]-1[[1-[3-[2-(7-chloro-2-quinolinyl) ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl]thio] methyl]cyclo propane acetic acid”</td>
<td>C_{35}H_{35}ClIN_{2}O_{3}.Na 608.18</td>
</tr>
<tr>
<td>Sr.No</td>
<td>Name</td>
<td>Chemical Structure</td>
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<td>Milnacipran HCl</td>
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<td>Dabigatran etexilate mesylate</td>
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<td>6</td>
<td>Ropinirole HCl</td>
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