Chapter-1

Introduction to drug impurities and their quantification in pharmaceutical dosage

1.1 Impurity quantification in pharmaceutical dosage forms

Identification and quantification of impurities in drug compounds is a crucial task in pharmaceutical process development for quality and safety. Related components are the impurities in pharmaceuticals which are unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during stability testing, or develop during formulation or upon aging of both API and formulated APIs in medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Various analytical methodologies were employed for the determination of related components in pharmaceuticals. There is a great need for development of new analytical methods for quality evaluation of new emerging drugs.

An impurity as defined by the ICH (*The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use)* guidelines is "any component of the medicinal product which is not the chemical entity defined as the active substance or an excipient in the product". Analytical methods for impurities estimation should be stability indicating to monitor the stability of pharmaceutical dosage forms during the investigational phase of drug development, and once the drug is marketed, the ongoing stability studies must be conducted/ performed. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enables to establish a retest period/shelf life for a drug substance and a recommended storage condition. Methods can be developed which measure the amount of drug remaining, the amount of drug lost (or the

appearance of degradation products), or both. The development of these methods for pharmaceuticals can be approached from several avenues. Related components, related substances, and related impurities are synonyms for the term impurities; the use of above terms at different phrases means one and the same (i.e. impurities).

1.2 Sources of impurities in pharmaceutical substances:

The origin of impurities in drugs is from various sources and phases of the synthetic process and preparation of pharmaceutical dosage forms. Majority of the impurities are characteristics of the synthetic route of the manufacturing process. There are several possibilities of synthesizing a drug; it is possible that the same product of different sources may give rise to different impurities. According to the ICH impurities are classified as organic impurities, inorganic impurities and residual solvents. Organic impurities may arise from starting materials, by products, synthetic intermediates and degradation products. Inorganic impurities may be derived from the manufacturing process and are normally known and identified as reagents, ligands, inorganic salts, heavy metals, catalysts, filter aids and charcoal etc. Residual solvents are the impurities introduced with solvents [1- 6]. Of the above three types, the number of 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 the side effects of the drug substances. The situation is different with the organic impurities. Drugs prepared by multi-step synthesis results in various impurities, their number and the variety of their structures are almost unlimited and highly dependent on the route and reaction conditions of the synthesis and several other factors such as the purity of the starting material, method of isolation, purification, conditions of storage etc. In addition, toxicity is

unknown or not easily predictable. For this reason the ICH guidelines set threshold limit above which the identification of the impurity is obligatory.

1.2.1 Sources of organic impurities:

Organic impurities may arise during the manufacturing process and/or storage of the drug substance. These impurities are derived from drug substance synthetic processes and degradation reactions in drug substances and drug products. The process (synthetic process) related impurities can be derived from starting materials, intermediates, reagents, ligands, and catalysts used in the chemical synthesis, as well as by-products from the side-reactions of the chemical synthesis. Degradation products are derived from the chemical degradation of drug substances and drug products under storage or stress conditions. They may be identified or unidentified, volatile or non-volatile, and include the following.

1.2.1.1 Impurities originating from drug substance synthetic processes:

Most of the drug substances (low molecular weight) are chemically synthesized. Chemical entities, other than the drug substance, that are involved or produced in the synthetic process can be carried over to the final drug substance as trace level impurities. These chemical entities include raw materials, intermediates, solvents, chemical reagents, catalysts, by-products, impurities present in the starting materials, and chemical entities formed from those starting material impurities (particularly those involved in the last steps of the synthesis). These impurities are usually referred to as process impurities [7]. The goal of process impurities identification is to determine the structures and origins of these impurities. This knowledge is critical for improving the synthetic chemical process, in order to eliminate or minimize process impurities [8].

1.2.1.2 Starting materials and intermediates:

Starting materials and intermediates are the chemical building blocks used to construct the final form of a drug substance. Unreacted starting materials and intermediates, particularly those involved in the last steps of the synthesis, can potentially survive the synthetic and purification process and appear in the final product as impurities [9,10]. For example, in the synthesis of tipranavir drug substance, aniline is the intermediate in the last step of the synthesis. Due to the similarity between the structures of aniline and the final product, it is difficult to totally eliminate it in the subsequent purification step. Consequently, it appears in the drug substance at around 0.1% [11].

1.2.1.3 Impurities in the starting materials:

Impurities present in the staring materials could follow the same reaction pathways as the starting material itself, and the reaction products could carry over to the final product as process impurities. Knowledge of the impurities in starting materials helps to identify related impurities in the final product, and to understand the formation mechanisms of these related process impurities. One such example is the presence of a 4-trifluoromethyl positional isomer in 3-trifluoromethyl-alpha-ethylbenzhydrol (flumecinol), due to the presence of 4-trifluoromethylbenzene impurity in the starting material, 3-trifluoromethylbenzene. A second example involves a 2-methyl analogue present as a trace impurity in tolperisone, due to the presence of 2-methylpropiophenone in the starting material, 4-methylpropiophenone.

1.2.1.4 Reagents, ligands and catalysts:

These chemicals are less commonly found in APIs; however, in some cases they may pose a problem as impurities. Chemical reagents, ligands, and catalysts used in the synthesis of a drug

substance can be carried over to the final products as trace level impurities. For example, carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (**CCMTHP**), which is used as an alkylating agent in the synthesis of a ß lactam drug substance, was observed in the final product as an impurity. Many chemical reactions are promoted by metal based catalysts. For instance, a Ziegler-Natta catalyst contains titanium, Grubb's catalyst contains ruthenium, and Adam's catalyst contains platinum. In some cases, reagents or catalysts may react with intermediates or final products to form by-products. Pyridine, a catalyst used in the course of synthesis of mazipredone, reacts with an intermediate to form a pyridinium impurity.

1.2.1.5 By-products of the synthesis:

All chemical reactions are not 100% selective; the side-reactions are common during the synthesis of drug substances. By-products from the side reactions are among the most common process impurities in drugs. By-products can be formed through a variety of side reactions, such as incomplete reaction, over reaction, isomerisation, dimerisation, rearrangement, or unwanted reactions of starting materials or intermediates with chemical reagents or catalysts.

1.2.1.6 Products of over-reaction:

In many cases the previous steps of the syntheses are not selective enough and the reagents attack the intermediate not only at the desired site. For e.g. in the synthesis of nanodralone decanoate, the last step of the synthesis is the decanoylation of the 17-OH group. In the course of overreaction the reagents also attack the 4-ene- 3 oxo group leading to an enol ester- type impurity (3, 17β- dihydroxyestra-3, 5- diene disdecanoate).

1.2.1.7 Products of side reactions:

Some of the frequently occurring side reactions (which are unavoidable in drug synthesis) are well- known to the synthetic chemist; other which lead to trace level impurities have to be

detected and elucidated during impurity profiling. The formation of diketopiperazine derivative is a typical side reaction in peptide synthesis.

1.2.1.8 Impurities originating from degradation of the drug substance:

Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. Degradation products resulting from storage or formulation to different dosage forms or aging are common impurities in the medicines. The definition of degradation product in the ICH guidelines is a molecule resulting from a chemical change in the substance brought about by overtime or due to the action of light, temperature, pH or water or by reaction with excipient and/or the intermediate container closure system [12]. For example in the case of aspartame, in the presence of moisture, hydrolysis occurs to form the degradation products L- aspartyl- L-Phenyalanine and 3-benzyl-6-carboxymethyl 2, 5-diketopierazine. A third degradation product β-L- aspartyl-L-phenylalanine methyl ester is also known to form. Aspartame degradation also occurs during prolonged heat treatment.

1.2.2 Enantiomeric impurities:

The majority of therapeutic chiral drugs used as pure enantiomers are natural products. The high level of enantio selectivity of their biosynthesis excludes the possibility of the presence of enantiomeric impurities. In the case of synthetic chiral drugs, the racemates which are usually marketed, if the pure enantiomer is administered, the antipode is considered to be an impurity. The reason for its presence can be either the incomplete enantio selectivity of the syntheses or incomplete resolution of the enantiomers of the racemate [13]. Although the ICH guidelines exclude enantiomeric impurities, pharmacopoeias consider them as ordinary impurities.

A single enantiomeric form of chiral drug is now considered as an improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index with a

more favourable reaction profile. However, the pharmacokinetic profile of levofloxacin (S-Isomeric form) and ofloxacin (R- isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), levalbuterol (R-albuterol) and esomeprazole (S-omeprazole).

Typical examples of drugs containing enantiomeric impurities are:

a) Dexchlorophenarmine maleate (R enantiomer impurity allowed < 0.5%)

b) Timolol maleate (R enantiomer impurity allowed <1%)

c) Clopidogrel sulphate (R enantiomer impurity allowed < 1%)

In general, an individual API may contain all of the above mentioned types of organic impurities varying from negligible to significant level.

1.3 Requirement for control of impurities:

Impurities often posses unwanted pharmacological or toxicological effects by which any benefits from their administration may be outweighed. Impurities will have different disastrous efficacy, different bioavailability, adverse effects and toxic effects. In case of chiral impurities one isomer may produce the desired therapeutic activities, while the other may be inactive or in worst cases, produce unwanted effects. For example consider the tragic case of the racemic drug of n-phthalyl-glumatic acid imide that was marketed in the 1960's as the sedative Thalidomide. Its thereapeutic activity resided exclusively in the R-(+)-enantiomer. It was discovered only after several hundred births of malformed infants that the S-(+)-enantiomer was teratogenic. It is not only that one enantiomer reacts and the other does not but also in some instances different enantiomers can have different effects as shown in Table 1.1.

Table: 1.1 Pharmaceutical products and their effect of chirality

Compound	Isomer	Effect
Thalidomide	S-isomer	Teratogenic
	R-isomer	Sleep inducing, anti-nausea
Barbiturates	S-isomer	Depressant
	R-isomer	Convulsant
Opirates	R,S-isomer	Narcotics
	S,R-isomer	Non-addictive cough mixture
Labetalol	S,R-isomer	Alpha-blocker
	R,R-isomer	Beta-blocker
Pencillamine	D-isomer	Anti-arthritic
	L-isomer	Toxic

1.3.1 Pharmacopoeial status:

The quality of a chemically active substance with respect to organic impurities is controlled by a set of tests within a pharmacopoeial monograph. Individual monographs are periodically updated to keep place with scientific progress and regulatory developments. Following the revised ICH Q3A (R2) impurity testing guidelines major pharmacopoeias will continue publishing new or revised relevant monographs and general chapters. Active substances found to contain an organic impurity not detected by the relevant pharmacopoeial tests prescribed below are not of pharmacopoeial quality, unless the amount and the nature of this impurity are compatible with GMP [14].

Two general chapters (<466> & <1086>) of the US Pharmacopoeia (USP) deal with organic impurity testing. Concepts and definitions are clearly described although different terminology from that of ICH is used. Until now, one of three types of tests in bulk pharmaceutical chemicals is ordered:

1. A chromatographic purity test coupled with a non- specific assay
2. A chromatographic purity- indicating method that also serves as an assay

3. A specific limit test for known impurities, a procedure that requires reference standards for these impurities [15].

In the future, new and revised USP individual monographs will include tests that actually control specified and unspecified organic impurities. Where different routes of synthesis yield different impurity profile, different analytical procedures will be proposed. All specified impurities will be separately limited, with a further limit of 0.10% for any unspecified (unknown) impurity. Total impurities above the disregard limit should be less than 1.0%. USP also proposes that a suitable test for detecting impurities that may have been introduced from extraneous sources should be employed in addition to tests provided in a specific monograph.

The European Commission decided that the principles and terminology of the revised ICH Q3A should be implemented in the European Pharmacopoeia (EP) monographs of the active substances; both new and already published [16]. A new general chapter concerning the control of impurities in pharmaceutical substances was introduced in the fifth edition of the EP, while a revision of the monograph entitled substances for pharmaceutical use has also been done. According to the policy of EP, control of the relevant organic impurities in synthetic drug substances is often accomplished by the test of related substances. Currently, it is a limit test (comparison of the peak areas), but will progressively be changed to utilize a quantitative acceptance criterion [17].

Some individual monographs already satisfy this demand. More tests are ordered, if the general test does not control a given impurity or there are other special reasons. Potential impurities with a defined structure that are known to be detected by the tests in a monograph, but are not known to be present in medicinal substances above the identification threshold, are referred to as detectable impurities. They are limited by a general acceptance criterion. EP

individual monographs published in the new format include a separate section in which all impurities (specified and detected) are listed. Unidentified specified impurities are not listed in this section, but their specific acceptance criteria along with appropriate analytical characteristics (e.g., retention time) are reported in the text, wherever it is applicable.

However, previous EP monographs were not having a related substances test in the new explicit style and are to be read and interpreted according to the recent amendments. During the coming years, EP individual monographs now published in the old format will be revised to contain related substances tests and lists on specified and other detectable impurities. Monographs containing tests for related substances based on TLC will also be revised.

1.3.2 ICH guidelines:

According to ICH guidelines, each impurity must be investigated with respect to both chemistry and safety aspects. The former include identification (structural characterization), reporting and quantitation using suitable analytical procedures, while the latter include a process of acquiring and evaluating data concerning the biological safety of an impurity (qualification). Individually listed impurities, limited with specific acceptance criteria, are referred to as specified and they can be either identified or unidentified.

Unspecified impurities are limited by a general acceptance criterion. A decision tree for the identification and qualification along with the corresponding thresholds, which are dependent on the maximum permitted daily dose (MDD), is given by ICH. Summing up, the following list of organic impurities must be presented in the specification of a synthetic drug substance:

- Each specified identified or unidentified impurity
- Any unspecified impurity

- Total impurity

Specified unidentified impurities are referred to by an appropriate qualitative analytical description (e.g. relative retention time) [18].The ICH topics, codes of quality guidelines are given in Table 1.2

Table: 1.2: ICH topics, codes of quality guidelines

Topics / Code	Quality guidelines
Q1A(R2)	Stability Testing of New Drug Substances and Products
Q1B	Stability Testing: Photo stability Testing of New Drug Substances and Products
Q1C	Stability Testing for New Dosage Forms Annex to the ICH Harmonised Tripartite Guideline on Stability Testing for New Drugs and Products
Q1D	Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products
Q1E	Evaluation of Stability Data
Q1F	Stability Data Package for Registration in Climatic Zones III and IV
Q2(R1)	Validation of Analytical Procedures: Text and Methodology
Q3A(R2)	Impurities in New Drug Substances
Q3B(R2)	Impurities in New Drug Products
Q3C(R4)	Impurities: Guideline for Residual Solvents
Q4B	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions
Q4B ANNEX 1	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Residue on Ignition/Sulphated Ash General Chapter
Q4B ANNEX 2	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Test for Extractable Volume of Parenteral Preparations General Chapter
Q4B ANNEX 3	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Test for Particulate Contamination: Sub-Visible Particles General Chapter
Q4B ANNEX 4A	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on MICROBIOLOGICAL EXAMINATION of Non-Sterile ProdMicrobial Enumerations Tests General Chapter
Q4B ANNEX 4B	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Microbiological Examination of Non-Sterile Products: Test for Specified Micro-Organisms General Chapter

Q4B ANNEX 4C	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Microbiological Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use General Chapter
Q4B ANNEX 6	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Uniformity of Dosage Units General Chapter
Q4B ANNEX 7	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Region on Dissolution Test General Chapter
Q4B ANNEX 8	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Sterility Test General Chapter
Q4B ANNEX 9	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Tablet Friability General Chapter
Q4B ANNEX 10	Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions on Polyacrylamide Gel Electrophoresis General Chapter
Q5A(R1)	Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin
Q5B	Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of R-DNA Derived Protein Products
Q5C	Quality of Biotechnological Products: Stability Testing of Biotechnological/ Biological Products
Q5D	Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products
Q5E	Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process
Q6A	Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances
Q6B	Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
Q7	Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients
Q8(R2)	Pharmaceutical Development
Q9	Quality Risk Management
Q10	Pharmaceutical Quality system

1.4 Control of organic impurities:

Control of the organic impurities in new drug substances is based on the maximum daily

dose and total daily intake (TDI) of the impurities. Table1.3 provides the ICH threshold for control of the organic impurities in new drug substances. Depending on whether the maximum daily dose is higher or lower than 2g, organic impurities in a new drug substance at (or greater than) 0.05% or 0.1% requires identification. Based on the maximum daily dose, the identification thresholds for organic impurities in new drug products are divided into four groups to give more consideration to low dose drug products. For most of the new drug products, the maximum daily dose is between 10mg–2g/day. Therefore, any impurities at 0.2% or greater would have to be identified.

Table: 1.3: Organic impurity threshold in new drug substances based on ICHQ3A

Reporting Thresholds	
Maximum Daily Dose	**Threshold**
≤ 1 g	0.1%
> 1 g	0.05%

Identification Thresholds	
Maximum Daily Dose	**Threshold**
< 1 mg	1.0% or 5 µg TDI, whichever is lower
1 mg - 10 mg	0.5% or 20 µg TDI, whichever is lower
>10 mg - 2 g	0.2% or 2 mg TDI, whichever is lower
> 2 g	0.10%

Qualification Thresholds	
Maximum Daily Dose	**Threshold**
< 10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg - 100 mg	0.5% or 200 µg
TDI, whichever is lower	>100 mg - 2 g
0.2% or 3 mg TDI, whichever is lower	> 2 g

1.5 Stability testing of new drug substances and drug products:

Analytical methods for impurities estimation should be stability indicating to monitor the stability of pharmaceutical dosage forms during the investigational phase of drug development,

and once the drug is marketed, for the ongoing stability studies which must be performed. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enables to establish a retest period/shelf life for a drug substance and a recommended storage condition. Methods can be developed which measure the amount of drug remaining, the amount of drug lost (or the appearance of degradation products), or both. The expert working group of the ICH developed a guideline on stability testing for registration and application within the European Union, Japan and the United States. The goal of the ICH stability guidelines was to exemplify the core stability data package required for new drug substances and products such that the data generated in any of the regions is mutually acceptable in the other two. The guidelines applys to the information required for the registration and applications of new molecular entities and drug products, but not to abbreviated or abridged applications, clinical trial applications, and so on. The test conditions are selected based on the climatic conditions in three areas so that test data provides evidence on the variation in quality with time under the influence of a variety of representative environmental factors. These data in turn allow recommended storage conditions and shelf lives to be established.

1.5.1 Drug substances:

The primary stability studies for the drug substance show that it will remain within specification during the retest period. Long-term (12-month) and accelerated (6-month) testing are performed on at least three batches. Batches can be prepared at a minimum of pilot scale, but

should use the same synthetic route and a method of manufacture that simulates the final process to be used at manufacturing scale. In addition, supporting stability on laboratory-scale batches may be submitted. The quality of the batches placed on stability should be representative of the quality of (a) Material used in preclinical and clinical studies and (b) Material to be made at a manufacturing scale.

The first three batches, made post approval should be placed on long-term stability using the product registration protocol. Testing should cover physical, chemical, and microbiological properties susceptible to change during storage and likely to affect the product quality, safety, and/or efficacy. Validated stability-indicating methods should be used. The number of replicates to be run depends on the results of validation studies, and the limits should be derived from material used in preclinical and clinical studies, including both individual and total upper limits for impurities and degradation products. The length of the studies and the storage conditions should cover storage, shipment, and subsequent use, although use of the same conditions as for the drug product will facilitate comparative review and assessment. Other conditions to be included should be scientifically justified. Temperature sensitive drugs should be stored at the labeled long-term storage temperature and accelerated testing should be conducted at 15°C above the designated long-term storage temperature with appropriate relative humidity conditions.

At the time of regulatory submission, a minimum of 12 months at 25± 2°C/60% RH ± 5 (long term) and 6 months at 40°C ± 2°C/75% RH 5% (accelerated) is required. If significant changes are noted at the elevated temperature, additional testing at an intermediate condition, such as 30± 2°C/65% RH ± 5% should be conducted. The registration application should include a minimum of 6 months of data from a 12-month study at the intermediate condition. Significant change at 40°C and 75% RH is defined as failure to meet the specification. Long term testing

should be continued to cover all retest periods. Normally, testing under long term conditions is performed every 3 months for the first year, every 6 months for the second year, and then annually. Containers employed in the long-term stability study should be the same or simulate the actual packaging used for storage and distribution. As the application is pending review, accumulated stability data should be submitted. Accelerated or intermediate temperature data may be used to support shipping conditions and evaluate the effect of short-term excursions outside the label storage conditions [19-20].

Because long-term stability is used to establish appropriate retest periods, it should be noted that the degree of inter batch variability affects the confidence that a future batch will remain within specifications for the entire retest period. As a rule, determination of the time at which the 95% one-sided confidence limit for the mean degradation curve intersects the acceptable lower specification limit is acceptable, combining data into one overall estimate to account for variability. Before combining the data, appropriate statistical tests should be applied to make sure it is allowable. If inappropriate to combine data, the retest period may depend on the minimum time to remain in specification. The nature of the degradation relationship determines the need for transformation of the data for linear regression analysis. This relationship can generally be fitted to a linear, quadratic, or cubic function on an arithmetic or logarithmic scale.

Statistical methods can be used to test the goodness of fit of the data on all batches and combined batches, where appropriate, to the assumed degradation curve. If the data show little degradation or variability, a retest period can be justified without statistical analysis and a limited extrapolation of real-time data may be undertaken when supported by the accelerated data. Any extrapolation must be justified, because it assumes that the same mechanism of degradation will

continue beyond the observed data; this evaluation should include assay, degradation products, and any other appropriate attributes.

The storage temperature range should be based on the stability data and used in accordance with the national or regional requirements. Specific labeling requirements should be stated, particularly for drugs that cannot freeze; terms such as ambient and room temperature should be avoided.

1.5.2 Drug Product:

The stability program for the drug product should be based on knowledge of the drug substance and experience from experimental and clinical formulations. Unless specifically noted in this section, the requirements for drug substances also apply to drug products. Accelerated and long-term data should be provided on three batches of the same formulation and dosage form in the containers and closure proposed for marketing. This revision of the ICH guidelines specify only solid oral dosage forms, and it states that two of the three batches placed on stability should be at least pilot scale, but that a third may be smaller, for example, 25,000-50,000 tablets or capsules. As with drug substance, at least 12 months of long-term stability data should be submitted at the time of regulatory filing. If possible it is also desirable to file the stability data of the finished products by the manufactures. Data on laboratory-scale batches of drug product is not acceptable as primary stability data, but may be submitted as supportive information, as the data on associated formulations or packaging. If required, preservative efficacy testing and assays on stored samples should be performed to determine the content and efficacy of antimicrobial preservatives. Differences between release and shelf-life specifications for antimicrobial preservatives should be supported by preservative efficacy testing. Limits for tests

such as dissolution and particle size require reference to results of bioavailability and clinical batches.

Storage at high relative humidity is important for solid oral dosage forms, but is not necessary for products such as solutions, suspension, and so on, stored in containers designed to provide a permanent water barrier. Low relative humidity (10-20%) is appropriate for products of high water content stored in semi permeable containers. Testing of unprotected drug product can be a useful part of stress testing and package evaluation, as studies in related packaging materials. If a product needs to be reconstituted or diluted, stability in the final form should also be addressed.

1.6 Scope and Objectives of research work:

The present research work focuses on the development of novel stability-indicating analytical methods for some active pharmaceutical ingredients and few of their dosage forms. The work also includes the validation of the developed methods as per ICH requirements and demonstrates the suitability of the HPLC methods to determine the related components in different classes of pharmaceutical compounds which include Tapentadol (pain relievers), Nebivolol hydrochloride (antihypertensive), Bendamustine hydrochloride (used for treating chronic lymphocytic leukemia), Haloperidol (antipsychotic) and Pitavastatin (used in treatment of blood cholesterol lowering). The developed methods were validated according to regulatory norms. The developed methods can be successfully implemented during the quality monitoring and also well employed for the assessment of quality during its storage.

References:

[1] Impurities in New Drug Substances, ICH Guidelines (Q3A).

[2] Gorog.sandor, chemical and analytical characterization of related organic impurities in drugs, *Analytical and Bioanalytical Chemistry*, **377**, 2003, 852-862.

[3] Rao. R. Nageswara , Nagaraju.V, An overview of the recent trends in development of HPLC methods for determination of impurities in drugs, *journal of pharmaceutical and*

biomedical analysis, **33**, 2003, 335-377.

[4] Grekas. Nikolaos, organic impurities in chemical drug substances, *pharmaceutical technology Europe,* 2005.

[5] Rousseau. Guy, international conference on harmonization impurity guidelines- the industry perspective, *drug information journal,***34,** 2000, 903-907.

[6] Roy.Jiban, Pharmaceutical impurities, *AAPS PharmSciTech*, **3,** 2000.

[7] Qiu, F. and Norwood, D.L., Identification of pharmaceutical impurities, *journal of liquid chromatography & related technologies*, **30**, 2007, 877-935.

[8] Gorog, identification and determination of impurities in drugs*, Journal of pharmaceutical and biomedical analysis,* **4**, 2000, 12-13.

[9] British Pharmacopoiea, Pharmacopoieal norms for enantiomeric impurities, 2001**.**

[10] Gavin & Olsen, a quality evaluation strategy for multi-source active pharmaceutical ingredient (API) starting materials**,** *Journal of Pharmaceutical and Biomedical Analysis,* **41**, 2006, 1251-1259.

[11] Muehlen, E. Impurities in starting materials and drugs**.** *Pharmazeut. Ind*. **4**, 1992, 837-841.

[12] ICH, harmonized tripartite guidelines on impurities in new drug products (Q3B).

[13] High beam encyclopedia, Organic impurities in chemical drug.

[14] USP Guideline for submitting a request for revision of the USP/NF. Chapter 1, non-complex drug substances and products.

[15] General chapters <466>, "Ordinary impurities" and <1086>, "Impurities in official articles," in USP 28-NF 23.

[16] CPMP Guideline on control of impurities of Pharmacopoeial substances, 2004.

[17] General chapter 5.10, control of impurities in substances for pharmaceutical use and general monograph**,** 2005.

[18] Dantus.Mauricio.M and Wells.L.Margaret, regulatory issues in hromatographic analysis in the pharmaceutical industry, *Journal of liquid chromatography & related technologies,* **27**, 2004, 1413-1442.

[19] Baertschi SW. The Role of Stress Testing in Pharmaceutical Product Development, presented at the American Association of Pharmaceutical Scientists, *midwest regional meeting, Chicago, IL,*1996.

[20] Weiser, developing analytical methods for stability testing. Pharma Technol, 1998, 20-29.