CHAPTER 1:

INTRODUCTION

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1.1Impurity profiling in pharmaceutical API

Pharmaceuticals are the compounds manufactured for use as medicinal drugs. The bulk API producing industry of well determined quality forms a vital part of all formulation related pharmaceutical industries. Over the last few decades much attention is paid towards the quality of pharmaceuticals that enter the market(1).

Due to the implementation of strict regulations by regulatory bodies, there continues an increase in product recalls from the market. One of the prime reasons for product recall is presence of impurities or degradation products (DPs) beyond the allowed standard limits. There are two factors which determine safety of pharmaceutical drug i) pharmacological to toxicological ratio of drug. ii) Impact of impurities present in drug on humans(2).

Impurities can be defined in broader term as “a molecule which is the result of degradation of drug substance by oxidation, deamination, proteolysis and many more chemical reactions the result of which could affect stability of drug product over a time”(3). Such impurities should be identified and quantified.

Impurities present in various pharmaceuticals can come from variety of sources: a) Impurity comes from raw materials; b) the manufacturing process adopted; c) due to instability of the product during processing and storage and at last; d) from the atmospheric contaminants during storage (4).

Impurity profiling helps in quantification and identification of impurities in pharmaceuticals and plays a major role in predicting and maintaining stability and efficacy of pharmaceutical agents. Since none of the regulatory bodies give clear definition about specific impurities, profiling can be termed as “the common name of analytical activities with an aim of detection, identification and/or elucidationof the structure and quantifying inorganic, organic or even solvent residues present in bulk drugs and pharmaceutical final products."

1.1.1 Regulatory Guidelines on Impurities in an Active Pharmaceutical Ingredient:

From ethical issues to competitive market along with that mainly safety and efficacy of product supports for the impurities monitoring in the pharmaceuticals. Therefore unified regulatory guidelines are necessary that everyone has to follow to address the queries related to impurities in pharmaceuticals. USFDA have been supports the guidelines which are prepared

under the ICH guidelines (5). The guidelines of the ICH has been developed by the joint efforts of the United States, European Union (EU) and Japan. ICH ensures that different NDA (New Drug Applications) and ANDA (Abbreviated New Drug Application) application have consistent requirements across the different regions.

ICH guideline of CTD (Common Technical Document) M4Q (R1)specify in Sections 3.2.S.3.2 and 3.2.P.5.5 the requirement of characterization of impurities in new drug substances in new drug products (6). Guidelines of European medical agency specify the requirement of impurity characterization in section CPMP/QWP/130/96 (i.e. Chemistry of New Active Substances) (7); EMEA/CHMP/CVMP/QWP/450653/2006 ((i.e. Assessment of Quality of Medicinal Products Containing Existing or Known Active Substances); CHMP/QWP/297/97 Rev 1 corr ((i.e. Summary of Requirements for Active Substances in the Quality Part of the Dossier) (8), etc.

The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines “stability testing of new drug substances and products"- Q1A
2. ICH guidelines “Impurities in New Drug Substances”- Q3A
3. ICH guidelines “Impurities in New Drug Products”- Q3B
4. ICH guidelines “Impurities: Guidelines for residual solvents”- Q3C
5. US-FDA guidelines “NDAs -Impurities in New Drug Substances”
6. US-FDA guidelines “ANDAs – Impurities in New Drug Substances”
7. Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia

As per ICH Q3A impurity guideline, impurity in a pharmaceutical drug substance is “any component of the drug substance that is not the chemical entity defined as the drug substance”(3) and as per ICH Q3B impurity guideline, impurity in a drug product is “any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product”.(5)

1.1.2 Classification of the impurities:

According to USP (United States Pharmacopoeia) impurities are classified into three sections

1. Impurities in Official Articles
2. Ordinary Impurities
3. Organic Volatile Impurities

According to ICH Q3A (3) and Q3B (9) guidelines pharmaceutical Impurities can be classified as following three categories:

1. Organic Impurities (Process and drug-related)
2. Inorganic Impurities (Reagents, ligands, catalysts)
3. Residual Solvents (Volatile solvents)

Organic impurities:

Organic impurities can be arising during the manufacturing/synthesis or during storage of pharmaceutical drug substances/products. Organic impurities may be starting materials, intermediates, by-products, DPs, reagents, ligands and catalysts. Since organic impurities can be volatile in nature thus, it can be identified or unidentified.

Inorganic impurities:

Inorganic impurities can also arise during manufacturing of bulk pharmaceuticals that include reagents, ligands, catalysts, heavy metals, other residual metals, inorganic salts, and materials like filter aids, charcoal. As water is widely used in pharmaceutical operation it can be a great source of heavy metals i.e. Mn, Ar, Mg, Cr, and other metal salts of Na and K. The heavy metals can act as catalyst for the oxidation and hydrolytic reactions in pharmaceuticals, resulting in their degradation. Generally inorganic impurities are known and are easily identified by performing limit tests.

Residual solvents:

Residual solvents are generally organic or inorganic solvents/media that are used during production of pharmaceuticals. It is very difficult to remove these residual solvents completely by the work-up process therefore solvents that are known to cause toxicity should be avoided in the production of pharmaceuticals. Residual solvents in pharmaceuticals are potentially undesirable substances as they either tend to modify the properties of certain pharmaceutical compounds or may prove hazardous when consumed by humans. The residual solvents are also known to affect physicochemical properties of the bulk drug substances such as crystallinity of bulk drug, which in turn may affect the dissolution properties, odor and color changes in finished products.

As per guidelines of ICH Q3C (10) the limits of residual solvent based on existing data of safety and toxicity. In Q3C term Q tands for the Quality, 3 is defining the 3rd number of quality guideline and C is the subdivision of the guideline and R refers to the revision and 7 denotes the number of revisions.

Residual solvents are classified in three categories:

Class 1 (Extremely toxic as well as hazardous) solvents : Solvents such as Benzene (Carcinogenic), Carbon tetrachloride (Toxic), 1,1 Dichloroethane (Toxic), 1,2 Dichloroethane (Toxic) are included and their concentration in pharmaceuticals is limited up to 2,4,8,5 ppm respectively. For environmentally hazardous solvents i.e trichloroethane a limit of 1500 ppm is applied.

During manufacturing of pharmaceuticals Class 1 solvents should be avoided. But if their presence is unavoidable, the definite concentration limit is applied, regardless of the actual patient intake dose.

Class 2 (Considerable less risky) Solvents: Class 2 solvent should be used up to 1500 ppm limit given by ICH for particular solvents. There are two different type of approaches mentioned in guidelines for limit setting of class 2 solvents. The first approach can use when PDD (permitted daily dose) is not known or estimated hence concentration limits are calculated on the basis of daily intake of theoretical product mass of 10g. The second approach is used when daily permitted dose of drug product is known and permissible concentration is decided on the basis of PDD.

Permitted dose exposure limit (PDE) is described for individual Class II solvents in this guideline according to their toxicity level and environmental hazard. However, PDE depends upon the no observed effect level or lowest observed effect level, some variability factors and several other factors. On the basis of PDE of the particular solvent and dose of the drug product, the allowable concentration of solvent in finished product can be determined. The relation between PDE and concentration in ppm is given below:

$$\text{Concentration (ppm)} = 1000 \text{ x } \frac{PDE}{Dose}$$

Here, PDE is given in terms of mg/day and dose is given in g/day.

Calculation for the concentration limit of the residual solvent in drug substance and drug product described with two different options in the guideline; depends on the dose of the drug substance or drug product.

Option-1 applies if the dose is not fixed, in that case consider the dose 10 g/day and ensure that the dose would not be exceeded over it so the calculation as per the option-1 is given below

Prototype calculation for the methanol as it is used in Imatinib mesylate Route of synthesis. The PDE for methanol is 30mg per day and dose of the drug substance is not fixed so consider highest dose 10g/day of the drug substance as per the guideline

$$\text{Concentration (ppm)} = 1000 \times \frac{30}{10}$$

$$\text{Concentration (ppm)} = 3000 \text{ ppm}$$

Similarly, the concentration limit of the other organic solvents involved in the synthesis of the drug substance is calculated.

Option-2 can be applied if the dose of the drug substance or drug product is fixed or known or dose is higher than the 10g/day. In this case the known dose incorporated in above equation and can get the concentration limit of the solvents.

Class 3 (Lower risk solvents):Class 3 solvents are limited up to 5000 ppm (0.5% w/w) in pharmaceuticals. Class 3 solvent are less toxic and possess lower risk to human health than class I or class II solvents. Some of the solvents are; Acetic acid, anisole, butanol, 2-butanol, isopropyl acetate, methyl acetate, butyl acetate, tert-butyl methyl ether.

Class 4 Solvents (Lowest risk solvents):For class IV solvents appropriate/enough toxicological data is not available hence the pharmaceutical manufacturers should justify the residual levels for these solvents in pharmaceutical products. The solvents under class IV are 1, 1-diethoxy propane, 1-1-dimethoxy propane, 2-2- dimethoxy propane, methyl isopropyl ketone, isooctane, isopropyl ether, methyl tetrahydrofuran, petroleum ether, trichloro acetic acid.

Now days, it is mandatory requirement in various pharmacopoeias to know the impurities present in APIs and finished drug products. Thus impurity profiling can act as a Quality Control tool. It can provide crucial data regarding the toxicity, safety, various limits of detection and limits of quantitation of several organic and inorganic impurities, usually accompany with APIs and finished products. There is strong requirement to have unique specifications/standards with regard to impurities.

Since impurities in drug substance could cause toxic effects in the patients, thus the guidelines on impurities in new drug substance (Q3AR2) have been issued by ICH.

1.2 Tyrosine kinase inhibitors

Tyrosine kinesis are group of enzymes that are responsible for the triggers of many protein synthesis in the body which is generally performed by process of signal transduction (11). Tyrosine kinase deactivated addition of phosphate group (phosphorylation) which is most important step in protein synthesis. Production of tyrosine kinase is directed by Abelson(Abl) gene present on chromosome 9 in humans.(12).

Chromosomal abnormality is the cause for 90% of CML(chronic myeloid leukemia) cases. This chromosomal abnormality was first discovered by scientist named Peter Nowell in 1960 and he found that chromosomal abnormality is a result of fusion between the Abelson (Abl) tyrosine kinase gene present at chromosome 9 and the break point cluster (Bcr) gene present at chromosome 22, leads to formation of chimeric gene (oncogene) (Bcr-Abl) which is implicated in CML pathogenesis(13).

Most tyrosine kinase inhibitors are used as antineoplastic drugs which is used to treat cancer. Tyrosine kinase inhibitors have proved very successful in treatment of chronic myeloid leukemia and acute lymphoblastic leukemia.

Tyrosine kinase inhibitors (TKIs) are a type of targeted therapy. TKIs are available as pills which can be taken orally(14). A targeted therapy identifies and attacks specific types of cancer cells while causing less damage to normal cells. In chronic myeloid leukemia (CML), TKIs target the abnormal BCR-ABL1 protein that causes uncontrolled CML cell growth and block its function, causing the CML cells to die(15). Tyrosine kinase inhibitors have proved very successful in treatment of in the chronic myeloid leukemia and acute lymphoblastic leukemia even in clinical studies (16).

1.2.1 Tyrosine kinase inhibitors

Till date total four TKIs drugs have been approved as first line therapy for chronic phase CML. These drugs are with registered marketed product name in parantheses.

- Imatinib mesylate (Gleevec®)
- Dasatinib (Sprycel®)
- Nilotinib (Tasigna®)
- Bosutinib (Bosulif®).

1.2.2 Imatinib Mesylate:

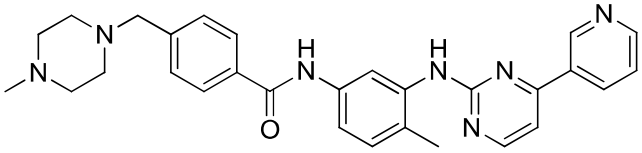

Fig 1.1 Structure of Imatinib Mesylate

Imatinib Mesylate is white amorphous powder which is chemically class 2-phenylaminopyrimidine derivative, designated chemically as 4-[(4-methylpiperazine-1-yl)methyl]-N-(4-methyl-3-(1) phenyl) benzamide-methane sulfonate (Fig.1.1). The drug Imatinib Mesylate comes under the pharmacological class of tyrosine kinase inhibitor.

Imatinib has been approved for the patients suffering from hematological malignancies or malignant sarcomas (17, 18). Imatinib is a specific inhibitor for a number of TK enzymes. It binds to the TK active site, leading to a decrease in kinase activity which plays a key role in the sarcoma tumor cell proliferation in GI track. Imatinib is rapidly absorbed orally and is highly bioavailable wherein 98% of an oral dose reaches the bloodstream.

Before Imatinib, GI- Sarcoma tumor is treated with resection. Resected GI-Sarcoma tumors can have high recurrence rate. Prior to availability of Imatinib, GIST (Gastrointestinal stromal tumor) patients had particularly high failure rate. Only 10% of patients remained disease-free after extended follow-up. Imatinib mesylate is a specific inhibitor of the tyrosine kinase in BCR-ABL +cell (19), given this high recurrence rate and the existence of an effective oral drug with a low toxicity profile which targets a tyrosine kinase (TK) expressed in over 95% of tumors. Pharmacological mechanism of action of Imatinib Mesylate is described in Fig.1.2

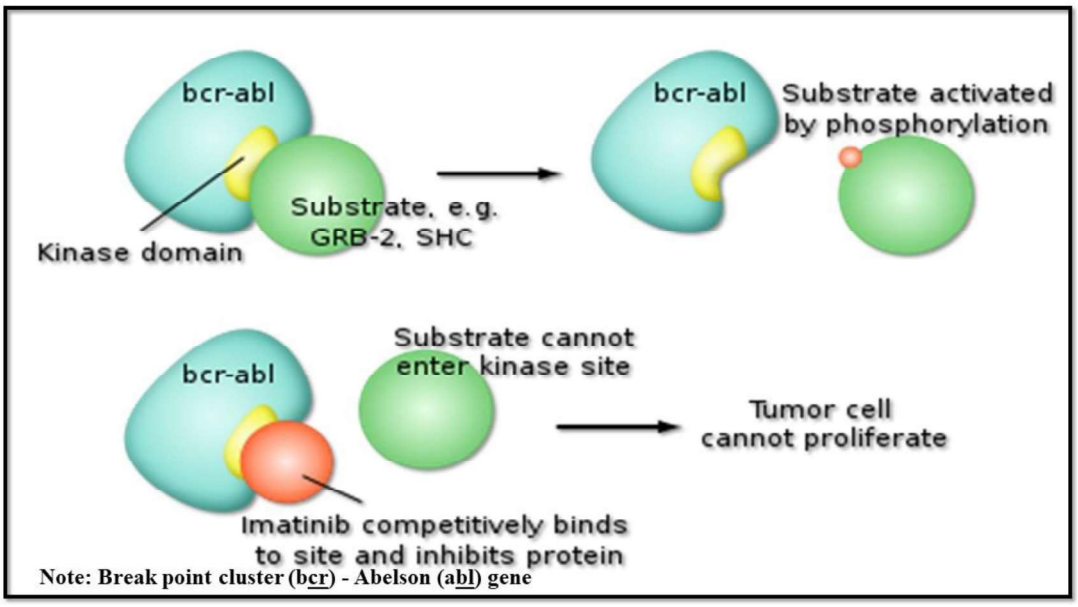

Fig 3.2 Mechanism of action of Imatinib mesylate in tumor cell

Imatinib mesylate is an approved drug for the treatment of gastrointestinal stromal tumor since 2002 (18) and for chronic myelogenous leukemia since 2011 (20) from the United States food and drug administration.

There are six solvents required during the process of the synthesis of Imatinib Mesylate drug substance. These six solvents are volatile in nature and hence can be determined through headspace gas chromatography in single analytical method. These six volatile organic solvents are methanol, acetone, dichloromethane, n-hexane, ethyl acetate and pyridine (21) and all these volatile organic solvents are required tobe identified and quantified during the manufacturing of the imatinib mesylate drug substance since most of the solvents are under the category of Class II solvents as per ICH guideline.

As per the ICH guideline the solvent residues must be controlled below their limit described in ICH guideline in finished drug substance of imatinib mesylate before approving the drug substance for the market.

Solvents used during the process of synthesis of Imatinib mesylate drug substance are given in table-3.1 with their class, permitted dose exposure and concentration limit according to the ICH guideline Q3C (R6).

Sr. no	Name of residual solvents in Imatinib mesylate	Class of solvent	Permitted daily exposure (mg/day)	ICH Limit (ppm)	Density (kg/m^3)
1	Methanol	II	30.0	3000	791.80
2	Acetone	III	50.0	5000	791.00
3	Dichloromethane	II	6.0	600	1326.00
4	n-Hexane	II	2.9	290	659.1
5	Ethyl acetate	III	50	5000	897.00
6	Pyridine	II	2.0	200	981.90

Table 3.1 Residual solvents used in Imatinib mesylate

So, the validated analytical method for the determination of residual solvents in Imatinib mesylate requires controlling the residual solvents below their ICH limit

1.2.3 Dasatinib

Dasatinib is an approved drug, sold under the brand name Sprycel. Dasatinib is an inhibitor of multiple tyrosine kinases. It inhibits the growth of chronic myeloid leukemia and acute lymphoblastic leukemia cell lines over expressing BCR break point cluster-ABL . Bcr-Abl tyrosine-kinase inhibitors (TKI) such as Dasatinib are considered as first-line treatment for the patients having chronic myelogenous leukemia (CML). Chromosomal abnormality is the cause for 90% of CML cases. This chromosomal abnormality was first discovered by scientist named Peter Nowell in 1960 and he found that chromosomal abnormality is a result of fusion between the Abelson (Abl) tyrosine kinase gene present at chromosome 9 and the break point cluster (Bcr) gene present at chromosome 22, leads to formation of chimeric gene (oncogene) (Bcr-Abl) which is implicated in CML pathogenesis. In vitro, Dasatinib is 325-fold more potent than Imatinib against cells expressing wild-type BCR-ABL(22).

Dasatinib is chemically described as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-l-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazole carboxamide(23). A recommended starting dose of Dasatinib for chronic phase CML is 100 mg once a day and for accelerated, myeloid or lymphoid blast phase CML or Philadelphia(Ph) chromosome-positive acute lymphocytic leukemia the CML dose is 70 mg twice a day (24).In clinical studies, treatment with Dasatinib was continued until disease progression or until no longer tolerated by the patient.

1.3 TGR5 agonist

Diabetes can be divided in two broadly defined categories: 1) insulin dependent diabetes mellitus 2) non-insulin dependent diabetes mellitus (25-27). In type 1 diabetes mellitus the insulin level in plasma was very low compared to normal value(28, 29). Patient below 30 years age are mainly suffering from type 1 diabetes mellitus(30). Insulin and its analogues are mainly used to treat type 1 diabetes(31, 32).Bile acids plays significant role in emulsification of lipids and absorption of fat soluble vitamins as a function of endocrine signaling system (33). Modification of distribution and signaling strategy of bile acid can lead to dramatic changes in glucose metabolism which includes shifts in satiety and glucose management (34).

Type 2 diabetes is a type of disease which is closely related with metabolism disorder of fat, carbohydrates and several types of proteins(35). Pharmacologically Type 2 diabetes is results of impaired insulin production, secretion or transport process to a target region (36). Number of antidiabetic drugs have been developed to reverse the insensitivity of muscles, liver and adipose tissue along with improved insulin release pattern in Type 2 diabetic patient till date (37). Incretin related substance, sulphonylureas and glinides are used widely for treatment of type 2 diabetes in clinical practice for impaired insulin secretion. From the above sulphonylureas are used most as antidiabetic drug worldwide (38). Injectable insulin products are also used for treatment but oral products hold the major ratio due to ease of medications and patient compliance. Various anti-diabetic agents are reported however unable to provide complete glycemic control hence there is high need for novel anti-diabetic control.

TGR5 (Takeda G-protein-coupled receptor 5) is identified as the first cell surface receptor activated by bile acids and this receptor is reported to mediate some of the endocrine functions of bile acids (39). This observation is in line with the fact that besides their role in dietary lipid absorption and cholesterol homeostasis, bile acids are also emerging as important and metabolic signaling molecules. The TGR5 receptor is expressed in numerous tissues, including the liver, gallbladder, ileum, colon, heart, spleen, kidney, placenta, lung, uterus, testis, mammary gland, prostate, skeletal muscle, brown adipose tissue, leukocytes, macrophages, endothelial cells and selected areas of the central nervous system. Most relevant in that context is that bile acids have been shown to increase energy expenditure in part through activation of mitochondrial function, hence preventing the development of obesity and insulin resistance in mice fed with high fat diet. This metabolic effect was shown to be mediated by TGR5 whose activation results in an increased intracellular activation of thyroid hormone, subsequently leading to an increase in energy expenditure (40). In enteroendocrine cells, it stimulates the release of both glucagon-like peptide-1 (GLP-1), influencing glucose

homeostasis. TGR5 receptors are activated by bile salts and it is present in gallbladder, brain, liver, spleen and intestine(41). As release of bile acids activate TGR-5 receptor which further induce release of glucagon like peptide form the enteroendocrine cell of intestine by increasing level of cAMP intracellular (42). Moreover, it also increases energy usage through the induction of type 2 iodothyronine deiodinase (D2) (40). Agrawal et al have reported various novel and potent TGR5 agonist which can be used as potential treatment of Type 2 diabetes (39). Thus, a novel TGR5 agonist ZY12201 may provide a treatment option for type 2 diabetes with simultaneous management of glucose levels, body weight, and associated complications. ZY12201 has the potential of becoming a good candidate for the development of new anti-diabetic drugs to be used in early stages of the treatment of metabolic diseases.

1.4 Objectives:

- To identify and characterize the process related and degradation related impurities in Dasatinib drug substance.
- To develop an accurate and highly sensitive reversed-phase liquid chromatography method for determination of process related and degradation impurities in Dasatinib in bulk drug substance.
- To develop and validate RP-HPLC method which is precise, economical and commercially viable quantitative determination of Dasatinib impurities and also be useful for industrial scale manufacturing.
- To develop degradation pathway and determine stability of Dasatinib drug substance in various conditions by performing force degradation study and generate a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
- To develop and validate headspace gas chromatography technique for the determination of organic volatile impurities in imatinib mesylate.
- To develop and validate an accurate and highly sensitive reversed-phase liquid chromatography-UV derivatization method for determination of genotoxic impurity hydrazine in imatinib mesylate drug substance.
- To develop an accurate reversed-phase liquid chromatography method for determination of process related impurity of novel TGR 5 agonist ZY12201.
- To elucidate structure of degradation products with resolution to stability related problems that can be helpful in production of more stable formulation and determining expiry date of novel compound ZY12201.

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