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Genotoxic Impurity Profiling of Imatinib Mesylate

[1]M. Rajavardhan Reddy*, [2]Dr. R. Suresh, [3]Dr.K. Kannan

[1]Research Scholar, Annamalai University, Tamil Nadu, India
[2]Department of Pharmaceutical Chemistry, Annamalai University, Tamil Nadu, India
[3]Head of the Department, Pharmaceutical Sciences, Annamalai University, Tamil Nadu, India

A B S T R A C T

Genotoxic impurities (GTIs) in pharmaceuticals are of increasing concerns to both pharmaceutical industries and regulatory agencies due to their carcinogenic potency for humans. Practical guidance with respect to the analytical determination of diverse classes of GTIs is currently lacking in the literature. This article provides an industrial perspective with regard to the analysis of GTIs that are commonly encountered during drug development. Determination of these impurities at ppm levels requires highly sensitive analytical methodologies, such as LC/MS, LC-MS/MS, RP-LC. The present review emphasized on the various methods used for the detection and quantification of genotoxic impurities.

Keywords: Imatinib Mesylate, genotoxic impurities, assay, method validation, anticancer drugs.

A R T I C L E I N F O

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***Corresponding Author**
M. Rajavardhan Reddy
Research Scholar,
Annamalai University,
Tamil Nadu, India
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1. Introduction

Genotoxic impurities induce genetic mutations, chromosomal rearrangements, chromosomal breaks and act as carcinogenic compounds. Genotoxicity deals with mutagensis, carcinogensis, teratogensis. Impurities present in active pharmaceutical ingredients responsible for deleterious action on a cell's genetic material affecting its integrity. Therefore, exposure to even low levels of such impurities present in final active pharmaceutical

ingredient (API) may be of significant toxicological concern. These compounds cause damage to DNA by different mechanism such as alkylation or other interactions that can lead to mutation of the genetic codes. Thus, the term "Genotoxic" is applied to those agents that interact with DNA and its associated cellular components (eg. the spindle apparatus) or enzymes (eg. topoisomerases) [1].

The genetic changes are responsible for heritable effects on germ cells and impose significant risk to future generations [2]. They cause alterations in the genetic material within living cells, which can be transmitted from one cell generation to another (somatic mutatios) or to the progeny of affected individuals through germ cells (germinal mutations). Origin of Genotoxic impurities in active pharmaceutical ingredients:

- Source of genotoxic impurities from starting materials, by products during its synthesis.
- Contaminants from packing material.
- Impurities formed by degradation due to aging or during manufacturing.
- Residual solvents are organic volatile chemicals used during manufacturing or impurities are formed during production.
- Heavy metals: Main source of heavy metals from water which is used in the process and the reactors (if stainless steel reactors are used), where acidification or acid hydrolysis takes place.
- Impurities are formed due to side reactions during the synthesis of drugs.

Drug substances (DS) process development and Drug product (DP) formulation development are two major areas of the drug development process. Impurities/degradants can be generated in either of the processes, from D S degradation or DS-Excipient interaction. These Impurities either non-genotoxic or genotoxic in nature. Regardless, they are regulated by food and drug Administrator (FDA)/ International conferences on Harmonization (ICH) guidelines. Routine impurity analysis in pharmaceuticals requires identification at levels of 0.05 percent to 0.2 percent depending on the daily dose. However, genotoxic impurities can be much harder to detect due to their presence at low ppm levels. This review concentrates on the regulations and analytical technologies used to detect and quantitate impurities (genotoxic and non-genotoxic) in pharmaceuticals [3].

Imatinib is a drug used to treat certain types of cancer. It is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and some other diseases. Imatinib is protein tyrosine kinase (PKT) inhibitor which potentially inhibits Abelson (Abl) tyrosine kinase in Vitro studies [1]. In this work we demonstrate the practical example for the analytical control of two genotoxic impurites in Imatinib mesylate. These impurities were observed to be process impurities. From the literature it was found out that these impurites are genotoxic [4]. The me t ho d is based on High performance liquid chromatography (HPLC) for determination of N-(2-methyl- 5-aminophenyl)-4-(3-pyridyl)-2-pyrimidine amine (i.e. Imp-A) and N-[4-methyl-3-(4-methyl-3-yl-pyrimidin-2-ylamino)- phenyl] -4- chloromethyl benzamide (i.e. Imp-B). The method was validated as per In ternat ional conference e o f Harmonization (ICH) guidelines in terms of limit of specificity, linearity accuracy, detection (LOD), Limit of quantitation (LOQ), precision and solution stability [5].

As novel synthesized drug, there are only few methods in the literature for Imatinib Mesylate quantification in pharmaceutical dosage forms and for its purity evaluation in bulk drug. It was approved by US Food and Drug Administration (FDA) in 2001. And it is recently official in European Pharmacopoeia as active substance only in April 2015.

Imatinib Mesylate is a protein-tyrosine kinase inhibitor. It is useful for the treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and some other diseases [6].

It is designated chemically as 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl] benzamidemethane sulfonate with empirical formula $C_{29}H_{31}N_7O.CH_4O_3S$. The usual tablet dose is 100 mg and 400 mg. Although genotoxic and carcinogenic properties can be acceptable for some active pharmaceutical ingredients depend upon clinical circumstances (e.g., cancer chemothrapies). These substances add significant risk without any benefit to the active pharmaceutical ingredients. Impurities in drug substances and drug products generally do not have any beneficial effects and may impose a risk without associated benefit. Hence, there is a need of suitable guidelines and commitment from pharmaceutical industries to address this issue in the drug substance or drug products. Genotoxic agents alter the structure, information content, segregation of DNA, including those which cause DNA damage by interfering with normal replication process. Components that include in genotoxic impurities are interact with DNA either directly or indirectly and modification in DNA will takes place eg. Alkylating agents, intercalating agents

Types of Genotoxic impurity:

1.Carcinogens

These are the agents which cause cancer by affecting the genome or disrupt the cellular processes. It can cause and facilitate the propagation of cancer. The mutagenic and clastogenic activity act as carcinogenicity. The risk of causing cancer is increased by altering the cellular metabolism or directly damaging DNA by carcinogenic agents. They induce the uncontrolled, malignant division, ultimately leading the formation of tumors due to interfering the processes in cells. Apoptosis is occurs due to severe damage of DNA. Carcinogens mainly classified into two groups such as genotoxic and non genotoxic. Genotoxins are the substances which cause irreversible genetic changes or mutations with binding to DNA. They are categories into

two types such as chemical agents and non-chemical agents. Some chemical agents such as N- nitroso-N-methyl urea and non- chemical agents such as ultraviolet light and ionizing radiation [7].

Non genotoxins agents affect the DNA by indirect way and they promote the growth. Some organic compounds and hormones are includes in non genotoxins. In 1965, established an international agency for research on cancer (IARC) which is an inter governmental agency a part of world health organization of the United Nations. In 1971, it has published a classification of possible carcinogens:

Group 1:

The agents which includes in groups. 1st are definitely carcinogenic to humans.

Group 2:

The agents are suspected to carcinogenic to humans.

Group3: These substances are not classifiable as a human carcinogen.

Group 4:

Substances is not suspected as a human carcinogen (http.//en.wikipedia.org/wiki/carcinogen)

2. Mutation

The irreversible change in DNA sequence of a genome is known as mutation. Mutation in DNA sequence can alter the sequence of amino acid of the protein encoded by the gene (learn.genetics.utah.edu). DNA made by smaller units but in long sequence strung together. Mainly four basic types of units: A, T, G and C. These letters represent the base of DNA sequence such as adenine, thymaine, guanine and cytosine. Even in large mutations the number of chromosomes will changes, where sequence of the DNA with in chromosomes break and then rearrange [8].

Assessment and control of genotoxic impurities:

These impurities could be limited to new applications for existing active substances where assessment of the route of synthesis, process control and impurity profile does not provide reasonable assurance that no new or higher level of genotoxic impurities are introduced. The pharmaceutical industries regulate it to recognize their respective obligation to limit genotoxic impurities.

Therefore, substantial efforts are made during development to control all impurities at safe concentrations [9]. Genotoxic impurity can be identified by different methods by already known genotoxic impurity, possessing the similar functional group with known genotoxic impurity, positive test by genotoxicity assay. Muller classified the genotoxic impurities into 5 groups:

Group 1: This impurity is more dangerous, it is known genotoxic carcinogens that need to be avoided as much as possible.

Group 2: The compounds that are genotoxic but with the unknown carcinogenicity and need to be controlled with a threshold of therapeutic concern (TTC) approach.

Group 3: These impurities have alerting structures that are different to the parent drug substance. The assessment of this genotoxic compound will place it into group 2 or as an ordinary impurities group 5.

Group 4: These impurities are parent related alerting structure in which the genotoxicity studies on the API have already been performed and applied to the related impurities.

Group 5: These impurities have no alerting structure or indication of genotoxic potential and considered ordinary impurities that falls within the scope of ICH guidelines [10].

Importance of genotoxic impurity:

During the synthesis of active pharmaceutical ingredients various reaction steps are involved for conversion of basic starting material to the final products. Various intermediate products are formed during the synthesis and reaction involves the byproducts, catalysts, solvents and reagents which act as impurity. Even low levels of these are present in the final product as an impurity. Some unwanted toxicities including genotoxicity and carcinogenicity are observed by some reactive chemicals and they may react with DNA bases causing mutations. Mutations can be rearrangement, breaks of chromosomes, covalent binding with DNA during replication. Genotoxic substances indirectly cause mutations by activating the cells. Various modifications in genetic material which can be caused by exposure to even very low level of genotoxins, can cause cancer. Due to these reasons, identification and control of genotoxic substances at very low levels are most important to ensure safety to the humen [11].

Regulatory Aspects:

The assessment of genotoxic impurities and determination of acceptable limits for such impurities is difficult in active substances. The EMEA guideline recognizes the limitations and proposes the use of a “Threshold of toxicological concern” (TTC) for genotoxic impurities. Genotoxic impurities arise during synthesis, purification and storage of new drug substance should be identified, based on a scientific appraisal of the chemical reactions involved in the synthesis. When a potential impurity contains structural alerts, additional genotoxicity, testing of impurity should be considered [12].

The EMEA recommends the acceptability of genotoxic impurities for which no threshold mechanism are identified for pharmaceutical evaluations. In general, pharmaceutical measurements should be guided by a policy of controlling levels “as low as reasonably practicable” (ALARP principle). A rationale of the proposed formulation strategy should be provided based on available formulation options and technologies. The reacting substance which show “alerting substances” in terms of genotoxicity which are not shared with the active substance should be considered [13].

In EMEA guidelines, a threshold of toxicological concern (TTC) has been developed to define the exposure level of any unstudied chemical that will not pose a risk of significant carcinogenicity or other toxic effects. TTC originally developed as a “Threshold of regulation” at the FDA for food materials was established based on the analysis of 343 carcinogens from a carcinogenic potency database. The TTC value was estimated to be 1.5µg/person/day. The concentration limit (in ppm) of

genotoxic impurity in drug substance derived from the TTC can be calculated based on the expected daily dose to the patient using equation:

Concentration limit (in ppm) TTC (g day) dose (g day) (http://www.emeu.eu.int)

Development Method

Chromatographic conditions and preparation of samples during the assay:

As we have mentioned before, we have used the Symmetry Shield column and then the Zorbax with a flow rate of 1.0 mL/min and a temperature maintained to 35°C. The injection volume was set to 15 µL and the detector was set at a wavelength of 267 nm. The composition of mobile phase was at the ratio of 60 % of the aqueous buffer solution and 40 % of acetonitrile. Stock solutions of Imatinib were prepared in methanol in the concentration of 0.1 mg/mL.

Chromatographic conditions and preparation of samples during the degradation of Imatinib Mesylate to its impurities:

For both columns, we proceeded such as for the assay with some modifications like the flow rate and the wavelength which were set at 0.8 mL/min and 234 nm, respectively. The gradient program was described below in Table 1. Stock solutions of Imatinib and its two impurities (Acid and Dimer) were prepared in methanol with a concentration of 1 mg/mL and 0.002 mg/mL, respectively [14].

3. Method Validation

The method validation was performed according to International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures. All samples were analyzed using the assay and the degradation chromatographic conditions.

Specificity:

Concerning the assay validation, specificity was evaluated by comparing the chromatograms and the data obtained from the diluent, the placebo solution, the standard solution (analyte: Imatinib Mesylate) and the test solution.

Linearity:

Linearity was investigated by preparing solutions at five spaced concentration levels which cover the interval of validation. This interval was represented by the concentrations of 60 %, 80 %, 100%120 % and 140 % for the validation of the assay and by 0.10 %, 0.20 %, 0.30 %, 0.40 %, and 0.50 % around the content accepted individual impurity limit (0.2 %) for the validation of the degradation method.

Precision: Repeatability and Intermediate Precision:

To determine the repeatability of the dosage, six representative test solutions with a concentration of 100% were prepared independently during the same day using the same equipment and with the same operator. The coefficient of repeatability was found to be equal to 1.108. The intermediate precision factor was equal to 1.451. It is based on precision study by changing one or more operating conditions and our parameter to change was the handling day. These calculated values attested the precision of the assay method.

Limit of detection

The sensitivity for detection can be demonstrated by determining the limit of detection (LOD). A signal to noise (S/N) ratio between 3 to 10 is generally considered to be acceptable for estimating detection limit. S/N ratios of individual peak were determined at different concentration at estimate LOD and respective %RSD was calculated for replicate injection (n = 3).

Limit of quantification

The quantification limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical S/N ratio of 10-30 is generally considered to be acceptable for estimating the limit of quantification. S/N rations of individual peaks were determined at different concentration to estimate limit of quantitation (LOQ) and respective % RSD was calculated for replicate injection (n = 6) [15].

4. Conclusion

GC-MS, LC-MS, HPLC, RP-LC, HPLC-MS, LC-MS-MS, RP- HPLC and RP-LC methods were specifically used for the detection and quantification of genotoxic impurities in drug Imatinib mesylate. The limit of detection (LOD) and limit of quantification (LOQ) were determined successfully by many researchers and it is suggested that the determination of genotoxic impurities is an essential step for analysis of active drug(s) so as to minimise their harmful effects.

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