Review On: Advanced Techniques for Detecting and Controlling Genotoxic Impurities

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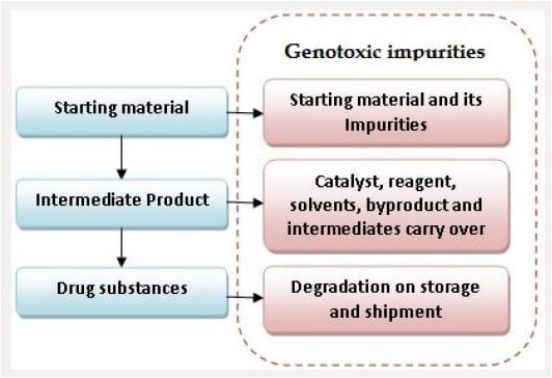
Abstract: **Genotoxic impurities (GIs) are known to be carcinogenic, making their management during the synthesis of pharmaceuticals crucial for ensuring the safe use of drugs, even at trace levels. The existence of DNA-reactive impurities in drug substances and products presents a considerable challenge for both drug regulators and the pharmaceutical industry. Numerous regulatory guidelines and position papers have been established to regulate the permissible levels of these impurities. This compilation provides updated information on GIs and reviews the regulatory considerations associated with them in active pharmaceutical ingredients and drug formulations. It also includes a thorough discussion of control strategies related to GIs. Analyzing GIs is a complex and demanding aspect of the drug development process, with the detection and quantification of these impurities at parts per million (ppm) or parts per billion (ppb) levels posing significant challenges for analysts. Consequently, various approaches for the analysis of GIs are also examined.**

Key Words: **Genotoxic impurities, Genotoxicity, Sources, Guidelines, Techniques for quantification**

INTRODUCTION

Definition: Genotoxicity refers to the harmful impact on the genetic material (DNA and RNA) within a cell, compromising its integrity. Substances that induce such effects are termed genotoxins, which can include mutagens—agents that cause mutations, such as radiation, chemicals, or physical factors. A genotoxic substance may also be classified as a carcinogen, a mutagen, or a teratogen, each associated with cancer, mutations, or birth defects, respectively. (1)

Sources of Genotoxicity: Genotoxic impurities can originate from multiple sources in drug compound production, with the primary source being the starting materials used in the manufacturing of drug substances and their associated impurities. Additionally, genotoxic intermediates and by-products generated during the manufacturing process may persist as contaminants in the final therapeutic product. Furthermore, solvents, catalysts, and reagents employed in the synthesis process can also introduce genotoxic contaminants into pharmacological compounds. (2) There are several ways that genotoxic contaminants might be included in a therapeutic ingredient, including the starting ingredient for the synthesis of medicinal resources are key sources, and its impurity. (3) Additionally, genotoxic intermediates and by-products generated during the synthesis process may also be transferred to the final drug substances as genotoxic impurities. (4)



Classification of genotoxic impurities:

Category	Definition	Guidance on Control of Human Exposure
Category 1	Precedent for mutagenicity and carcinogenicity	PDE, TTC, or staged TTC
Category 2	Mutagens with unknown carcinogenic potential or a “close-in” structural analog	TTC or staged TTC
Category 3	Alerting structure—unique and unknown mutagenic potential	TTC, staged TTC, or test in Ames assay
Category 4	Alerting structure—Non-unique and qualified in comparison to API	ICH Q3 controls apply

Category 5	No structural alerting features	ICH Q3 controls apply

Table no1: Classes of genotoxic impurities(5)

Significance:

The possibility of mutation, chromosomal breakage, and chromosomal rearrangements can be decreased by identifying genotoxic contaminants. Establishing the GTI limit is a crucial need for safe medication development. (6) A small amount of GTI present during pharmaceutical product manufacture can result in serious genetic issues. The inclusion of GTI in a final product makes it very difficult for regulatory agencies to approve a medicine. (5)

Techniques for genotoxic impurities:

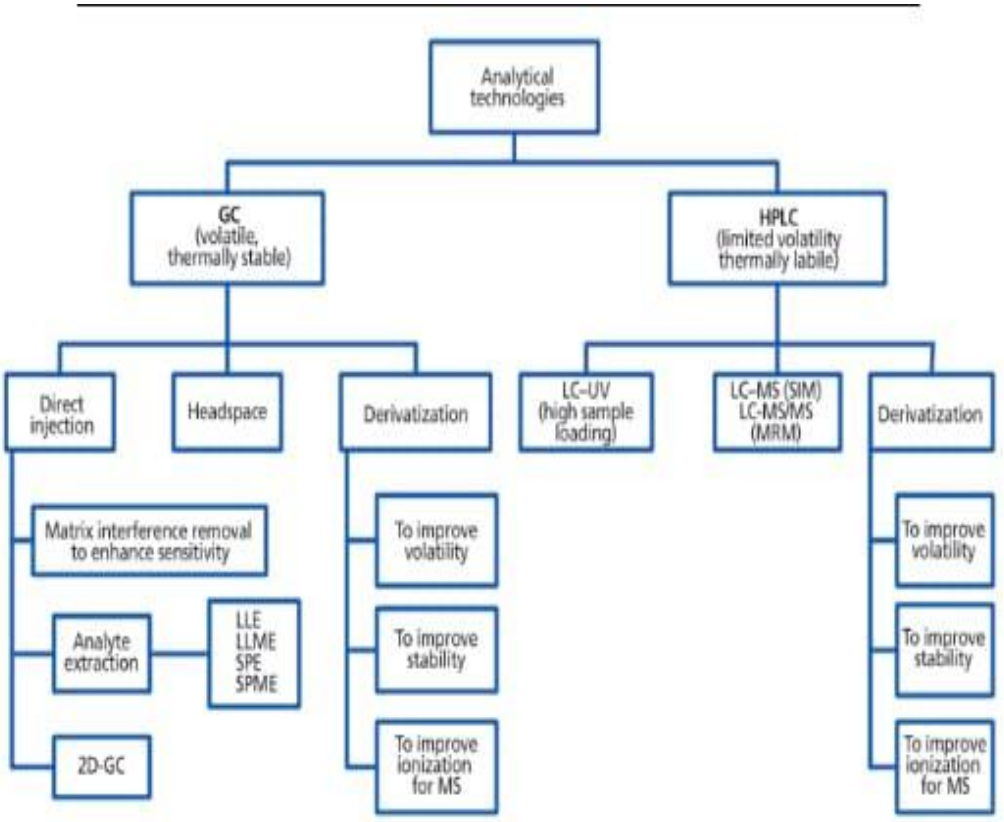


Fig: Genotoxic Impurities Analysis by Profacgen

1. Reversed-Phase High-Performance Liquid Chromatography (RPLC) – This is the most commonly employed technique, particularly effective for the separation and identification of non-volatile glycosides, including alkyl benzenesulfonates and sulfonate contaminants.

2. High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV): This technique is used to analyze alkylating contaminants and associated substances in medications like atorvastatin and verapamil. Although it works well, its sensitivity may occasionally be reduced.

3. Alkylating contaminants are commonly detected using High-Performance Liquid Chromatography Combined with Mass Spectrometry (HPLC-MS), a technology that provides increased sensitivity.

4. Hydrophilic Interaction Liquid Chromatography (HILIC) – This method is particularly effective for identifying polar impurities that pose challenges when analyzed through RPLC.

5. High-Performance Liquid Chromatography with Evaporative Light Scattering Detection (HPLC-ELSD) – This technique provides greater sensitivity than UV detection for certain impurities, particularly in intricate samples such as herbal medicines.

6. Gas Chromatography (GC) with Electron Capture Detection (ECD) – This method is employed for the

detection of specific volatile impurities but is not applicable for non-volatile active pharmaceutical ingredients (APIs). (7)

Gas Chromatography (GC) for Identifying Genotoxic Impurities (GTIs):

1. Volatility-Based Detection – GC is utilized to detect GTIs by evaluating their volatility properties, allowing for the differentiation between volatile and non-volatile compounds.
2. Sample Introduction Methods – There are two common approaches for introducing samples:

a) Liquid Injection – The sample is directly injected in its liquid state.

b) Headspace Injection – This technique is preferred for volatile impurities as it reduces contamination risks and improves measurement precision.

3. Headspace Injection Process – The sample is dissolved in an appropriate solvent (such as water, DMSO, NMP, or DMF), heated in a sealed vial, and the generated gas (headspace) is then injected into the GC.
4. Advantages of Headspace Injection – This method minimizes contamination risks, avoids the introduction of non-volatile substances into the system, and enhances the overall efficacy of the analysis.
5. Limitations – Some analytes require liquid injection due to their difficulty in evaporation or their sensitivity to elevated temperatures. (8)

Non chromatographic techniques:

Capillary Electrophoresis (CE) is recognized as a highly effective method for analyzing genotoxic impurities, characterized by its exceptional separation efficiency. The technique's capability to process minimal sample volumes and deliver swift analytical results enhances its appeal in the realm of pharmaceutical quality control.(9)

Detecting Genotoxic Impurities (GIs) Through NMR:

1. Advantages of NMR

Facilitates the identification of chemical structures of impurities.

Enables quantitative analysis without the need for calibration.

Requires minimal sample preparation.

2. Challenges Faced

Exhibits low sensitivity for trace-level impurities.

Potential for signal interference with the primary drug.

Demands high concentrations of samples.

3. Strategies to Enhance Sensitivity

Utilize higher magnetic field strengths (e.g., 600 MHz instead of 400 MHz).

Implement cryogenic probes to improve signal detection.

Elevate sample concentration to enhance detection thresholds.

Employ selective excitation techniques to concentrate on impurity signals.

Utilize ^{19}F NMR for the analysis of fluorinated impurities.

4. Illustrative Applications

^{1}H NMR successfully identified an aldehyde impurity in a pharmaceutical sample.

^{19}F NMR detected a fluorinated impurity at concentrations as low as 10 ppm using optimized techniques. (9)

Control and Elimination Strategies:

Usually, genotoxic impurities can be identified by any of the following methods.

(i) Already known genotoxins
(ii) possessing similar functional groups with known genotoxins,
(iii) testing positive by genotoxicity assays,
(iv) marked as a potential genotoxin by one of many computer-based structure–activity software programs.

The main focus of the risk assessment is to monitor the impurities that arise, particularly in the penultimate and final synthetic stages. If the genotoxic impurity is formed during the API synthesis, elimination may be achieved by changing the synthetic route and reagents used. However, this may not be feasible as the API synthesis is quite complicated and limited based on the chemistry and available reagents, making these approaches impractical. Another means of genotoxic impurity control in this situation is the implementation of additional purification steps in the synthetic route to get rid of the impurities. However, this is not effective if the genotoxic impurity forms after the API synthesis (e.g., API degradation or reactions with excipients/containers). Hence, the impurity levels still need to be measured and monitored in pharmaceuticals.(10)

At GlaxoSmithKline, improving quality assurance and guaranteeing reliable production processes require the development of a control plan based on quality-by-design principles. By moving the control points upstream in the process, this document describes the method used to create a control strategy that aims to achieve effective process control while

also potentially eliminating end product testing for critical quality attributes (CQAs) of drug substances.

This strategy is particularly beneficial for managing genotoxic impurities, given the complexities involved in creating and validating methods to detect trace amounts of these impurities. The approach involves implementing a series or combination of control elements at designated points that align with specific process steps or unit operations, thereby regulating the CQAs of the product and ensuring compliance with patient safety and efficacy standards. The control elements can be classified into three distinct modes:

(i) Attribute controls, which encompass in-process controls (IPCs) and specifications for starting materials, intermediates, solvents, and drug substances.

(ii) Parametric controls, which pertain to maintaining operations within proven acceptable ranges (PARs) for quality-critical process parameters (QCPPs) and quality process parameters (QPPs) that are associated with CQAs.

(iii) Procedural controls, which detail operations related to CQAs, including facility setup, equipment configuration, order of addition, selection of reagents and solvents, and the sequence of operations. (11)

CONCLUSION

The presence of genotoxic impurities (GIs) in pharmaceutical products poses significant risks to human health, including the potential for genetic mutations, chromosomal damage, and cancer. Given their dangerous nature, stringent regulatory standards have been implemented to control and reduce their occurrence in active pharmaceutical ingredients (APIs) and drug formulations. The pharmaceutical industry must utilize advanced analytical techniques, such as HPLC, GC, and NMR, to detect and quantify these impurities at very low levels. It is crucial to adopt various risk assessment and control measures, including modifications to synthetic routes, purification techniques, and quality-by-design (QbD) approaches, to ensure that GIs are maintained within acceptable thresholds. Categorizing GIs based on their mutagenic and carcinogenic characteristics aids in identifying the most effective control measures. Regulatory agencies like ICH and FDA have developed frameworks that support the identification, assessment, and management of GIs, ensuring that pharmaceutical products meet stringent safety criteria before reaching consumers. Moreover, integrating preventive strategies during the initial stages of drug development, optimizing reaction conditions, and selecting safer reagents and solvents can greatly reduce the formation of GIs.

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