INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Review Article

Analytical Research & Development on Genotoxic Impurities

Vishakha Pawar*, Pallavi Pawar, Akanksha Gangurde, Pankaj Aher, Mitesh Sonawane, Avish Maru

Loknete Dr. J D Pawar College of Pharmacy, Kalwan, Nashik, India

ARTICLE INFO

Published: 13 Oct 2025
Keywords:
Genotoxic Impurities, Guidelines, ICH, Threshold, Carcinogenicity, Impurities.
DOI:
10.5281/zenodo.17341882

ABSTRACT

Genotoxic impurities (GTIs) pharmaceutical products may pose a considerable risk to patients safety due to their potential to interact with DNA and induce mutations, carcinogenicity, or other adverse effects. These impurities often arise during active pharmaceutical ingredient (API) synthesis from raw materials, intermediates, solvents, catalysts, or by-products. Their detection and control represent a critical challenge in drug development because of their structural diversity, instability, and the need for ultra-trace quantification. Regulatory authorities, including the ICH, USFDA, and EMA, have established stringent guidelines to identify, monitor, and control GTIs, with techniques like the Threshold of Toxicological Concern (TTC) and classification of impurities depends on mutagenic potential. Analytical advancements, particularly in Chromatographic methods including GC, LC-MS, UPLC, and HPLC have greatly improved analytical sensitivity, allowing more accurate detection and quantification of compounds, and robustness for GTI detection at ppm levels. Complementary strategies, including in-silico assessments, structural alerts, and toxicological evaluations, further enhance impurity profiling. This review highlights the sources, classifications, regulatory perspectives, assessment strategies, and analytical methodologies vital to guarantee pharmaceutical products' efficacy, safety, and quality, it is essential to minimize risks associated with genotoxic impurities (GTIs).

INTRODUCTION

The identification, purity, physical characteristics, efficacy, bioavailability, and stability of drugs must be accurately determined through reliable analytical techniques. In pharmaceutical research, especially when assessing active pharmaceutical ingredients (APIs), developing and validating analytical methods is essential. This process ensures that the selected techniques are reliable, accurate, and appropriate for their specific purpose. These methods involve the analysis of specific properties of the compounds and

***Corresponding Author:** Vishakha Pawar
Address: *Loknete Dr. J D Pawar College of Pharmacy, Kalwan, Nashik, India*
Email : vishakhapawar554@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

comparison with established acceptance criteria. Thus, method development in analysis involves selecting and optimizing the most precise techniques to determine a drug's composition effectively [13]. Genotoxic impurities may enter drug substances from multiple sources, primarily from starting materials used during synthesis and their associated genotoxic intermediates or process related by-products. Moreover, solvents, catalysts, and reagents used during the synthesis process may also lead to the development of genotoxic contaminants in medicinal products. [7].

Impurity profiling during the development process by mainly regulated by guidelines set forth by the ICH and FDA. Among these, the ICH quality guidelines are considered highly comprehensive and detailed, serving as a key reference in pharmaceutical development [16]. Assessing genotoxic impurities (GIs) presents multiple challenges, including their detection, identification, quantification, and characterization. Additional complications arise due to the diverse chemical structures of impurities and the potential instability or reactivity of GIs. Therefore, it is essential to implement appropriate strategies, techniques, or tools to effectively detect, monitor, and manage these impurities. The use of advanced analytical instruments can significantly minimize difficulties during detection, allowing for the identification and measurement of a wider range of compounds, thereby improving the overall quality of analytical data. These technologies also enable the detection of impurities at levels even below current analytical thresholds [4].

However, the creation of genotoxic compounds while active pharmaceutical ingredients (APIs) are being produced follows specific pathways and is not a random event. These substances are often intentionally involved in the chemical processes due to their functional roles—whether used in stoichiometric or catalytic quantities, as solvents, or as by-products of the reactions. Their presence is typically managed throughout the API production stages, which commonly include multiple steps of intermediate isolation and purification that help eliminate most genotoxic impurities (GTIs) along with other contaminants. Moreover, the synthetic pathways initially developed for drug production are frequently refined by modifying reaction steps or optimizing conditions. These enhancements are intended to increase yield, improve selectivity, and ensure efficient use of materials, ultimately reducing the formation of unwanted by-products and residual reactants [16].

2.1Classification of genotoxic impurities

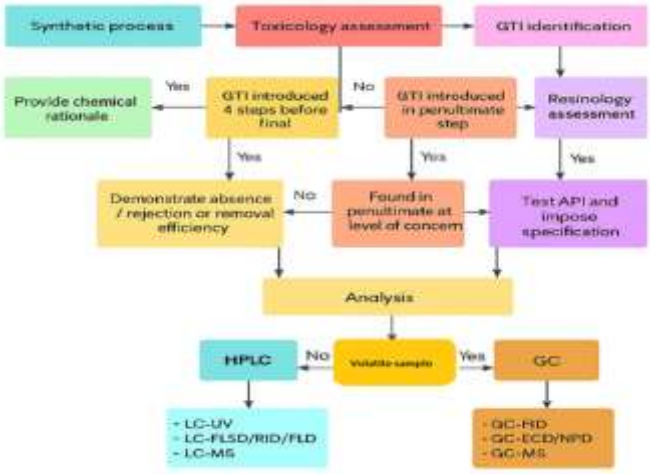

Fig 1. Classification of genotoxic impurities [7].

2.1.1: Alkyl halide.

Methyl, ethyl, and propyl halides are commonly employed as alkylating agents in industrial processes.These substances can react with important biological molecules like proteins and DNA by direct alkylation. However, the exact mechanisms behind their toxic effects are still not completely understood. Apart from their intentional use, alkyl halides may also arise as by-products in API processes, for instance, through reactions between alcohol-based solvents and hydrogen halides or via the DE quaternization of ammonium salts. [1].

2.1.2: Dialkyl Sulfates

In pharmaceutical formulations, methyl and ethyl derivatives of dialkyl sulfates are the most commonly applied, although the ethyl compound has also been employed as a chemical warfare agent. A strong methylating agent, dimethyl sulfate (DMS) can add a methyl group to atoms with lone electron pairs, such as those of oxygen, nitrogen, carbon, sulphur, phosphorus, and some metals. DMS is frequently used because it reacts faster and has fewer undesirable by products than methylating agents based on alkyl halides [1].

2.1.3: Hydrazine's

The harmful effects of hydrazine and its derivatives are primarily associated with the generation of reactive intermediates, such include oxygen-focused radicals, carbon-based radicals, and carbohydrate radicals. These unstable species can cause DNA alkylation and various forms of DNA damage. In pharmaceutical manufacturing, hydrazines hold significant importance—for example, they are used in the synthesis of sildenafil (Viagra), a widely recognized medication for erectile dysfunction, where hydrazine assists in forming a substituted pyrazole ring. A comparable transformation occurs in the preparation of the topoisomerase inhibitor sedoxantrone, during which a phenolic intermediate undergoes condensation with a substituted hydrazine to yield a pyrazole structure. Although the exact sequence of reaction steps has not been fully determined, it is considered likely that a hydrazone forms first, followed by chlorine displacement via the second nitrogen atom, ultimately resulting in closure of the pyrazole ring.[2].

2.1.4 Aromatic Amines

Generally speaking, neither fundamental nor supplementary aromatic chemical compounds are directly genotoxic. For them to transform into reactive electrophilic intermediates that can interact with biological molecules, metabolic activation is typically necessary. An S9 metabolic mix is typically needed to provide a good result in Ames tests (especially in strains TA98 and TA100).Some compounds, Certain nitro-substituted amines, 2,4-diaminotoluene, and 2,4-diaminoethylbenzene are among the compounds that can function as direct mutagens.

The primary metabolic processes for aromatic amines include ring oxidation, N-acetylation, and N-oxidation. The latter leads to the formation of N-hydroxy derivatives that undergo conjugation with acetate, sulphate, or glucuronide. Subsequent deconjugation generates a nitrenium ion (ArN+H), considered the key mutagenic or carcinogenic species that interacts with DNA.

Additionally, heterocyclic nitrogen compounds are converted into nitrogen-hydroxylamine intermediates by the enzyme nitro reductase, which is present in strains of Salmonella used in reversing evolution studies. This explains why many, though not all, nitroaromatic compounds exhibit mutagenicity for instance, nitrobenzene

tests negative in Ames assays, whereas 2,4-dinitrotoluene tests positive. In one case, an Ames-positive nitroaromatic compound being developed as an anticonvulsant was further evaluated with an extensive test panel, showing little likelihood of genotoxicity in vivo.[3].

2.1.5: Epoxides

Epoxides are the simplest members of the cyclic ether family, containing a three-membered ring. Because of the significant ring strain, they are highly reactive and frequently employed as intermediates in active pharmaceutical ingredient (API) synthesis. Their strained structure makes them likely to undergo ring-opening reactions with various nucleophiles such as compounds such as halogens, and ethanol, organometallic reagents, Sulfates, cyanide, and aromatic compound systems, and active methylene groups. However, this same reactivity underlies their genotoxic potential, since the electrophilic carbons in the epoxide ring can readily interact with nucleophilic sites in DNA, leading to alkylation. Substituted epoxides, such as glycidol Pharmaceutical synthesis frequently uses epichlorohydrin (1-chloro-2,3-epoxypropane), 2,3-epoxypropanol and 1, 2-epoxy-3- butene as building blocks. These derivatives typically undergo nucleophilic attack at the less sterically hindered carbon atom, although substituents that stabilize positive charge (e.g., aromatic or vinyl groups) can make both ring carbons susceptible to reaction. Because of their possible genotoxic effects, recent guidance, such as that provided by Elder and colleagues, emphasizes the importance of monitoring and controlling these impurities at trace levels during drug development.[16].

2.1.6: Sulphonate esters and their salts.

Sulfonate salts are frequently incorporated into pharmaceutical compounds and are widely used in drug synthesis. Their inclusion is often linked to improved biopharmaceutical performance and therapeutic benefits during formulation development. Converting active pharmaceutical ingredients (APIs) into their sulfonate salt forms enhances stability, often resulting in higher melting points. This salt formation also contributes to improved physicochemical properties such as solubility and stability, offering advantages in in-vivo performance.

Mesylate salts, for example, rarely form hydrates compared to salts derived from strong acids, but they can present genotoxic concerns during processes such as wet granulation. APIs that normally exhibit plastic deformation and lower melting points can achieve greater stability and elevated melting points upon salt conversion, which in turn increases crystal lattice energy. Sulfonic acid salts are particularly valuable due to their ability to enhance solubility, despite their inherently high melting points.

An illustrative case is haloperidol mesylate, which shows rapid dissolution (less than 2 minutes) in simulated gastric fluid (pH 2) because of its increased surface area and solubility, beyond what is explained by common ion effects. However, sulfonic acids can react with lower alcohols to form esters that function as alkylating agents, introducing potential risks of genotoxicity and carcinogenicity. Therefore, while sulfonate groups are advantageous for drug development, modern formulations must carefully address their safety implications.[17].

2.1.7: Nitrosamines

Nitrosamines, also referred to as N-nitrosamines, are compounds containing the nitroso group (N–NO). These substances are polar, hydrophilic, and uncharged, with high vapor pressure and excellent water solubility. Belonging to a broad class of N-

nitroso compounds, nitrosamines are recognized as highly potent carcinogens. They are generally produced from alkyl, alkaryl, aryl, or cyclic amines. A related category, N-nitrosamides, arises from N-alkylureas, N-alkyl carbamates, and simple N-alkyl amides.

Nitrosamine contaminants in pharmaceuticals raise serious safety concerns since they are considered potential mutagens, teratogens, and carcinogens. According to the ICH M7 (R1) guideline, nitrosamine impurities fall under Class 1 genotoxic impurities, meaning they are well established as mutagenic and carcinogenic based on animal studies and mutagenicity testing. Through mechanisms such chromosomal breakage, rearrangements, covalent bonding, or incorporation into DNA during replication, these pollutants can damage the genome by producing mutations. Even minimal exposure to nitrosamine impurities may trigger cancer development. Therefore, their detection at trace levels in drug products is critical to ensure patient safety [2].

3: Regulatory aspects.

Numerous guidelines and regulatory documents have been created to govern the levels of possible genetic contaminants in pharmaceutical products. Regulatory bodies and industry organizations have established particular standards to address these harmful contaminants. Institutions like the USFDA, ICH, and EMA have emphasized the dangers linked to PGIs in active pharmaceutical ingredients (APIs) and provided suggestions to reduce their occurrence. In response, research and development teams are expected to identify PGIs at early stages of process development, implement suitable analytical methods, and design control strategies for synthetic pathways that may produce such impurities. To keep patients safe, the allowed levels of these contaminants in drug levels need to be set below the established qualification thresholds. [8] The guideline titled "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk" outlines the rules for identifying, characterizing, assessing the level of toxicity and managing genotoxic impurities (GTIs) that may form during manufacturing or storage processes. [7].

3.1: FDA guidelines.

In December 2008, the USFDA released a prototype guideline titled “Genotoxic and Carcinogenic Impurities in Drug Substances and Products – Recommended Approaches.” This document was later superseded by the ICH M7 guideline, “Assessment and Control of DNA Reactive Impurities (PGIs) in Pharmaceuticals to Limit Potential Carcinogenic Risk.” The FDA’s guidance offered detailed recommendations for the safety evaluation of impurities that are known or suspected to be genotoxic. [8]. The US FDA has issued several important guidelines relevant to impurity profiling in the context of drug creation of both shortened applications for new drugs and novel drug applications (NDAs).Two key guidelines include:

3.1.1: Guidance for Industry: NDAs- Impurities in Drug Substances (February 2000): This guideline outlines requirements for impurity information in NDA submissions and Type II Drug Master Files associated with NDAs. It specifically excludes its applicability to biological and herbal products.

3.1.2 Guidance for Industry: ANDAs - Impurities in Drug Substances (February 2005): Intended for generic drug submissions, this document details the expectations to discover, qualify, and report contaminants.It describes the data needed for the Chemistry, Manufacturing, and Controls (CMC) section. This includes

contaminants in the active ingredients and breakdown products in the final mixture [15].

3.2: ICH guideline.

According to ICH guidelinescontaminants in pharmaceutical ingredients and goods must be carefully monitored to ensure patient safety. For example, ICH Q3A provides guidance on contaminants in novel medication ingredients, specifying limits for their assessing, determining, and recording. Similarly, ICH Q3B16 and ICH Q3C17 pertain to impurities in newly developed pharmaceuticals, particularly focusing on residual solvents. Solvents used in pharmaceutical manufacturing are grouped into three groups according to their possible health hazards. Class I solvents are considered highly toxic and should be strictly avoided whenever possible. Class II solvents pose some risk, so their use is allowed only within defined safety limits, known as permitted daily exposures (PDEs). Class III solvents, on the other hand, are considered to have low toxicity and are generally safe if daily exposure stays under 50 mg. In addition to solvent safety, the ICH Q3D guideline is being developed to help manage elemental impurities, such as heavy metals, by setting acceptable limits to ensure product safety and patient health [7].

Table 1: ICH quality guidelines.[1]

Threshold for APIs: Thresholds	**Daily maximum dosage**	
	≤2 g/day	≤2 g/day
The threshold for reporting	0.05%	0.03%
The threshold for identification	0.10% or 1.0 mg per day intake (whichever is lower	0.05%
The threshold for qualification	0.15% or 1.0 mg per day intake (whichever is lower)	0.05%

3.3: European Medicines Agency (EMA)

The European Medicines Agency (EMA) has outlined a framework and practical strategies for managing genotoxic impurities in new active pharmaceutical compounds. According to its guidance, a toxicological threshold of concern (TTC) has been established at 1.5 µg per day for genotoxic impurities. This value corresponds to an appropriate lifetime cancer risk of 1 in 100,000 for most drugs. Based on the projected daily dosage, the allowable limit for a genotoxic contaminant in a chemical used in drugs is often determined by applying a Threshold of Toxicological Concern (TTC). However, in certain cases especially for treatments of shorter duration regulators may permit higher limits, provided they are justified scientifically (European Medicines Agency / CHMP, 2006) [1].

A substance is considered genotoxic if it gives positive results in well-established genotoxicity in vivo or in vitro tests, particularly when there is indication that it can directly interact with DNA. In cases where in vivo data are unavailable, substances that test positive in vitro are usually treated as potential in vivo mutagens and carcinogens.

The guideline further categorizes genotoxic impurities into two groups, depending on the mechanism of action and the presence or absence of a threshold effect:

1. While genotoxic compounds are traditionally associated with non-threshold, linear dose-response relationships—where even minimal exposure could potentially cause DNA damage—some compounds have shown evidence of operating through a threshold-based mechanism. This means that below a certain exposure level, no significant genotoxic effects are observed, as the body's

natural defence and repair systems can effectively manage the damage.

2. Some genotoxic compounds have not shown clear experimental proof that there's a safe exposure level below which they don't cause harm. [6].

4. Genotoxic assessments.

Analytical scientists often use the terms "genotoxic" and "mutagenic" interchangeably, but they actually differ in their specific biological effects. Mutagenicity refers specifically to changes or mutations at the gene or chromosomal level. In contrast, genotoxicity encompasses a broader range of harmful interactions, including damage to the DNA helix and other cellular components involved in genetic material. [17].

Genotoxic impurities are typically identified using one or more of the following approaches: (i) recognition as previously known genotoxic substances, (ii) the presence of functional groups similar to those found among recognized genotoxins, (iii) positive results in genotoxicity testing assays, or (iv) prediction as potential genotoxins through computational structure–activity relationship (SAR) software tools [6].

Pharmaceutical scientists and toxicologists must perform toxicological evaluations to identify genotoxic impurities (GTIs), understand how they are introduced during the synthesis process, explore methods for their elimination, and establish acceptable limits in accordance with safety and regulatory guidelines. These assessments can involve both reviewing existing literature and using computational toxicology tools. The idea of using structural alerts to predict genotoxicity was first proposed by Ashby and Tennant, who linked electrophilic properties to DNA reactivity based on findings from Ames tests. Numerous structural alerts have since been reported in scientific literature. Commonly used software for such evaluations includes MDL-QSAR, MC4PC, and DEREK. [1].

In rodent studies that assess genotoxicity or cancer risk in living organisms, researchers typically use the maximum tolerated dose (MTD) or the highest dose that can realistically be administered. This approach helps reduce the risk of false-negative outcomes. Administering high doses helps compensate for the relatively limited number of animals used in these studies (usually 50 of each sex per group). However, for substances that are not genotoxic, or for genotoxic agents that are quickly metabolized and detoxified, using such high doses can lead to two major issues: Cytotoxic effects, which can trigger tissue damage and regenerative cell proliferation, Metabolic overload, which may overwhelm normal clearance systems and result in abnormal high-dose metabolism, potentially generating harmful by products like free radicals [3].

4.1: Approaches to identification and specification of genotoxic impurities.

When an impurity is detected in a drug substance or product, and it is a recognized substance with a structural genetic toxicology notice, existing Ames test results could already be available to the general population. If such information is lacking, or if the substance is new (for instance, confirmed through resources like pub chem), it is generally considered prudent to assume potential genotoxicity and manage it accordingly most often by applying 1.5 µg/day as the standard Threshold of Toxicological Concern (TTC) or alternatively, by conducting an Ames test. If the Ames assay indicates a positive response, the following strategies can be adopted:

- Control the impurity at the TTC level.

- Search for other pertinent information in the public domain, such as rodent bioassay findings.
- Carry out further genotoxicity evaluations through a compound-specific testing program [3].

In pharmaceutical analysis, conventional techniques such as HPLC coupled with UV detection (commonly applied to non-volatile compounds) and GC with flame ionization detection (suitable for volatile small molecules) are generally considered the primary tools for PGI assessment. However, these methods often lack the sensitivity needed to accurately quantify impurities at very low ppm concentrations, largely due to the nature of the analytes and the complexity of the sample matrices. Over recent years, significant advancements have been made in developing highly sensitive analytical approaches to enable reliable detection and quantification of PGIs in pharmaceutical products.[6]. There is a strong association (around 90%) between structural alerts and DNA reactivity. Genotoxic agents that function as electrophiles typically form covalent bonds with nitrogen and oxygen sites in DNA. Common electrophilic structural alerts include species such as carbonium ions, nitrenium ions, oxonium ions, epoxides, aldehydes, Michael acceptors (α,β-unsaturated ketones), peroxides, free radicals, and acylating agents. The predictive value of each alert varies; for instance, the toxicity of α,β-unsaturated ketones depends heavily on their specific chemical structure.

According to a white paper issued by the Pharma group, impurities are categorized into five groups based on genotoxic potential:

- **Class 1:** contaminants that have been shown to be cancerous and genotoxic, supported by reliable evidence of genotoxic mechanisms, with proven animal carcinogenicity and known human relevance.
- **Class 2:** contaminants that have the potential to cause cancer but are genotoxic (mutagenic), as they test positive in genotoxic assays yet lack carcinogenicity data.
- **Class 3:** Unrelated to the active pharmaceutical ingredient (API), impurities with structural warnings, with no direct genotoxicity data, but flagged based on structural features associated with DNA reactivity.
- **Class 4:** Impurities structurally related to the API that include a functional the parent was informed of the alarm drug.
- **Class 5:** Impurities without structural alerts or with sufficient supporting evidence indicating absence of genotoxic risk; these fall under the scope of ICH Q3A, Q3B, and Q3C guidelines.[15].

Pharmaceutical companies can reduce the likelihood that patients will be exposed to dangerous amounts of genotoxic impurities (GTIs) by proactively identifying and managing risks throughout the early phases of drug development. To support this, an in-silico genotoxicity assessment of drug substances and their starting materials should be carried out using specialized prediction tools such as Derek Nexus, Sarah Nexus, and Lhasa. These evaluations follow the framework of the ICH M7 guideline, which addresses the “Assessment and Control of DNA-Reactive (Mutagenic) Impurities in Pharmaceuticals to Reduce Potential Carcinogenic Risk.” Any impurity suspected of mutagenic activity must be evaluated and categorized in line with ICH M7 principles to determine its genotoxic potential.[13].

4.1.1: TTC Approach

The EMEA, or European Medicines Evaluation Agency, created the Threshold of Toxicological Concern (TTC) principle to determine permissible limits for genotoxic impurities (GIs) in pharmaceutical formulations. According to this concept, a daily intake of up to 1.5 µg of GIs is considered permissible. This limit is particularly applied in situations where carcinogenicity data for pharmaceutical substances are unavailable, such as for impurities in Classes 2 and 3. The permissible level of a specific GI can be calculated using the formula:

Limit = 1.5 µg/day ÷ maximum daily dose

The EMEA framework also incorporates a staged TTC model, emphasizing that the duration of exposure plays a critical role in cancer risk.[4].

5. Analysis of genotoxic impurities.

Analyzing impurities with extremely sensitive, robust, and selective analytical techniques is crucial to developing successful GTI control programs. The target requirements and expected impurity levels needed to satisfy regulatory standards determine the methodology to be used. The analytical methods should ideally be able to identify contaminants at concentrations ranging from 1 to 5 ppm (0.0001 to 0.0005% w/w). Given that many more organic pollutants, including excipients, may exist at even lower levels, these trace levels demand not only highly sensitive instruments but also methods with exceptional selectivity. Furthermore, the presence of a significantly higher concentration of the active pharmaceutical ingredient (API) can make it more difficult to detect low-level contaminants. Such contaminants are commonly analyzed using gas chromatography (GC) and liquid chromatography (LC), frequently in conjunction with mass spectrometry (MS). Examples of trace-level impurity analysis using GC and LC are commonly employed, and the use of online reaction monitoring is also explored for its potential benefits.[18].

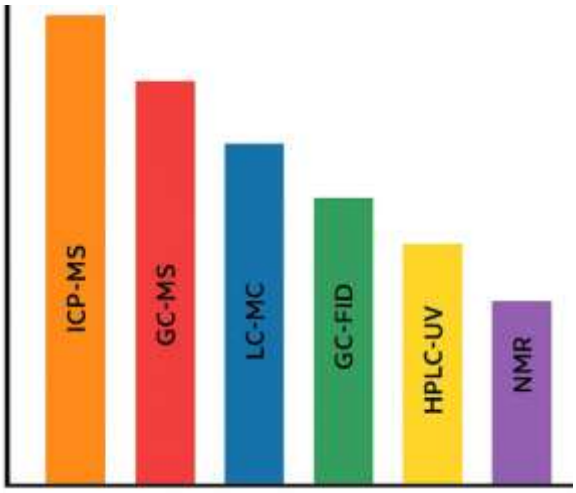

Fig.2: Analysis of GTI.[18].

5.1: HPLC

High-performance liquid chromatography (HPLC) is commonly employed to identify impurities in both raw drug substances and finished pharmaceutical products. It provides valuable information about the structural characteristics of impurities. HPLC supports a wide variety of column packing materials, detection techniques, and solvent systems, allowing for enhanced separation selectivity. Today, HPLC is often combined with multiple detectors such as ultraviolet (UV), fluorescence, mass spectroscopy, ultraviolet (UV), refractive index and evaporating scattering of light to assist in detecting and characterizing impurities. Due to its convenience and broad availability, HPLC paired with UV detection is considered the preferred method for assessing genotoxic impurities (GIs) [5].

Several validated HPLC methods for analysing GIs are reported in scientific literature. For example, Wang et al. (2016) described a straightforward and sensitive reversed-phase (RP) liquid chromatography approach using a UV detector to measure trace amounts of hydrazine in

pharmaceutical materials. Soni and Sanjay developed a Meloxicam and paracetamol are simultaneously estimated using the RP-HPLC technique with a photodiode-array detector, and their associated impurities in bulk and combination dosage forms. Additionally, Dousa et al. proposed an RP-HPLC method using a fluorescence detector for the analysis of impurities in vortioxetine.

To make sure that contaminants are successfully detected, the HPLC procedure can be optimized by using columns of various kinds, lengths, diameters, and particle sizes. Furthermore, specialized software tools are available to assist in optimizing chromatographic conditions to achieve efficient separation of impurities.[4]

5.2: Liquid Chromatography (LC)

When compared to normal-phase chromatography, reversed-phase (RP) HPLC is the most often used separation technique for non-volatile genotoxic substances (GTIs), which are commonly studied using HPLC. For the separation of pharmaceutical precursors, intermediates, and finished products active pharmaceutical ingredients (APIs), a large range of stationary phases are available and are used successfully. Different mobile phase compositions have been used, and gradient elution is frequently chosen over isocratic techniques. Binary systems that combine an aqueous phase with a less polar organic solvent, like methanol or acetonitrile, are still often utilized, nonetheless. During gradient runs, mild buffers such as phosphoric or acetic acid are frequently used to keep the pH at a suitable level. Even though acetonitrile-based buffer solutions are used often, extended exposure to acidic conditions can shorten the lifespan of columns and corrode HPLC pump parts, like piston seals, especially at high potassium phosphate concentrations. Some researchers choose gradient elution systems made entirely of methanol, acetonitrile, and water in order to get around these problems; they completely avoid using acidic buffers.

Effective distinction between the analyse peak the primary molecule is crucial, irrespective of the detector being utilized, because GTIs are usually present at ppm levels inside a matrix that is dominated by a large concentration of API. Therefore, the same guidelines that are used in drug-related impurity analysis should be followed when choosing columns and chromatographic settings for GTI analysis.

Hydrophilic interaction liquid chromatography (HILIC), a new addition to RP-HPLC, is particularly useful for sorting and retaining small, polar molecules. HILIC combines a stationary phase that is polar with a period of organic mobility, often acetonitrile with a little amount of water. The layer that the low water content develops on the stationary phase, makes it easier to separate polar analytes. Furthermore, mechanisms like ion exchange and hydrogen bonding may significantly facilitate the separation process.

HILIC provides enhanced retention for highly polar analytes, which may not be achievable with RP-HPLC columns. However, it is important to ensure that sample diluents are not overly aqueous, as high water content can impair the keeping polar analytes in place.

Ultra-performance liquid chromatography (UPLC) has emerged as a leading analytical technique for GTI analysis in recent years. UPLC uses narrower columns and operates at higher pressures than conventional HPLC, resulting in improved resolution, sensitivity, and analysis speed. In swine kidney samples, this method has been effectively used to isolate four

nitroimidazoles and three associated metabolites. Additionally, Kaufmann et al. used UPLC to analyze veterinary medications, such as seven nitroimidazoles in meat samples, in 15 minutes, providing a notable time benefit over conventional techniques that need longer runtimes. [5]

5.3: Gas Chromatography

Gas chromatography (GC) is a method used to measure volatile genotoxic impurities (GTIs) in drug substances. The stationary phase consists of a column filled with microscopic layers of liquid or polymer coated on a firm, inert support. The carrier gas is its moving phase. typically helium used for transporting the sample during analysis. For GTI determination, injection techniques such as direct liquid injection and headspace sampling are commonly employed. The sensitivity required for the method is determined by the concentration limits calculated according to the active pharmaceutical ingredient's (API) daily dosage, which also guides the choice of detector for accurate analysis [6].

In order to prepare the sample for headspace analysis, it must be in a solvent that is hot to the touch, such as water, dimethyl sulfoxide (DMSO), N-methyl pyrrolidone (NMP), or N,N-dimethylformamide (DMF), inside a sealed vial. During a regulated incubation period until equilibrium is reached, the volatile chemicals partition into the vial's headspace after heating. After that, the headspace's vapor is extracted and added to the GC apparatus for examination. One of the main advantages of headspace injection is that it lowers the possibility of contamination in the injector and the column by allowing only volatile substances, such as the target analytes, to enter the injector. Another key feature of this technique is that non-volatile drug substances (APIs) remain excluded from the column since they do not undergo partitioning into the headspace [5].

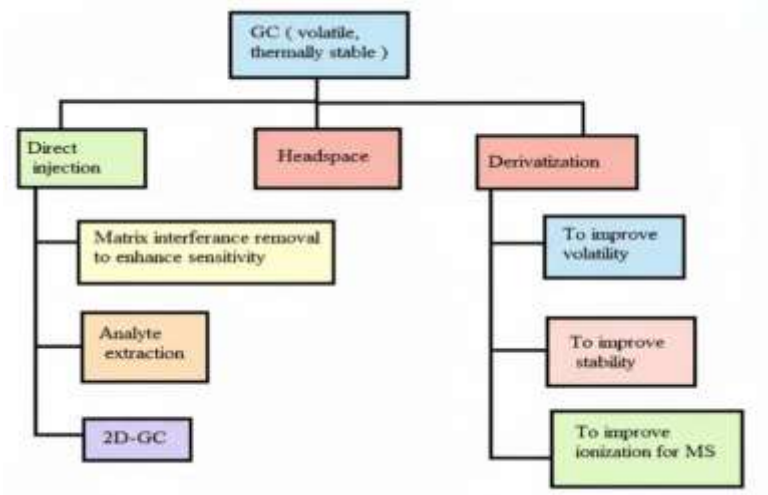

Fig.3. Gas chromatography technique for GTI analysis.[6]

CONCLUSION

The control of genotoxic impurities remains one of the most demanding aspects of pharmaceutical research and development. Their presence, even at trace levels, can significantly compromise patient safety, necessitating rigorous identification, quantification, and regulation. International guidelines, such as ICH M7, provide a strong framework for risk assessment, limit establishment, and control strategies. Analytical innovations—ranging from conventional HPLC and GC to advanced LC-MS and UPLC—have greatly enhanced the ability to detect and monitor

GTIs at levels consistent with regulatory expectations. Moreover, the integration of computational toxicology tools and in-silico predictive models has strengthened early-stage impurity assessment. To ensure long-term safety and efficacy of pharmaceuticals, it is vital to apply a proactive and multidisciplinary approach that combines synthetic optimization, advanced analytical methods, and strict adherence to global regulatory standards. Continued improvements in analytical sensitivity and predictive toxicology will further refine impurity control, supporting the development of safer and more reliable drug products. It gives primary information to control toxic level.

REFERENCES

1. Shaikh T. Impurities characterization in pharmaceuticals: A review [Internet]. SSRN; 2019. Available from: https://ssrn.com/abstract=3958603
2. Patel HB, Dave R, Vadariya S, Hirpara H. A brief review on genotoxic impurities in pharmaceuticals for the identification, control and determination in drug substances: A review.
3. Snodin DJ. Genotoxic impurities: from structural alerts to qualification. Org Process Res Dev. 2010;14(4):960–76.
4. Vandamme SS, Selvakumar K, Rajesh R. A review on analytical challenges in monitoring and controlling genotoxic impurities. Asian J Pharm Clin Res. 2020;13(10):10–5.
5. Al Azzam KM, Aboul-Enein HY. Recent advances in analysis of hazardous genotoxic impurities in pharmaceuticals by HPLC, GC, and CE. J Liq Chromatogr Relat Technol. 2016;39(1):1–7.
6. Reddy AVB, Jaafar J, Umar K, Majid ZA, Aris AB, Talib J, et al. Identification, control strategies, and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: a comprehensive review. J Sep Sci. 2015;38(5):764–79.
7. Gangu S, Laxmi VBG, Anusha S, Rathod A, Tadikonda RR, Boggula N. Genotoxic impurities from pharmaceutical drugs – A review. Int J Pharm Health Res. 2014;2(1):1–7.
8. Kalidindi VR, Somalanka BM, Seethamraju SM, Nori LP. Pharmaceutical impurities and their regulatory aspects with a special focus on genotoxic impurities. International J Pharm Res Allied Sci. 2024;13(2):1–15.
9. Honma M. Risk assessment and management of genotoxic impurities in pharmaceuticals. Mutagenesis. 2013;28(4):399–404.
10. Regulska K, Michalak M, Murias M, Stanisz B. Genotoxic impurities in pharmaceutical products: regulatory, toxicological and pharmaceutical considerations. J Med Sci. 2021;90(1):e502.
11. Alluri C, Naramsetti KK, Sharma G. Sensitive and selective analytical method for the quantification of two potential genotoxic impurities in azilsartan drug substance by using LC-MS/MS with multiple reaction monitoring (MRM mode). J Pharm Res Int. 2021;33(63A):467–78.
12. Singh D, Isharani R. A detailed review on analytical methods to manage the impurities in drug substances. Open Access Libr J. 2023;10(8):1–18.
13. Halder S, Narayana PV. A comprehensive review of current and emerging analytical techniques for the identification, quantification, and assessment of genotoxic impurities in drug substances.
14. Shallan AI, Abdel-Hakim A, Hammad MA, Abou El-Alamin MM. Spectrofluorimetric determination of selected genotoxic impurities in pharmaceutical raw materials

and final products. Sci Rep. 2022;12(1):15319.

15. Roy A, Nyarady S, Matchett M. Genotoxic and non-genotoxic impurities in pharmaceuticals: current regulations, status and trend. Chim Oggi. 2009;27(2):46–50.
16. Szekely G, Amores de Sousa MC, Gil M, Castelo Ferreira F, Heggie W. Genotoxic impurities in pharmaceutical manufacturing: sources, regulations, and mitigation. Chem Rev. 2015;115(16):8182–229.
17. Jadhav DR, Sonune KN. Genotoxic impurities and impurity profiling of pharmaceutical drug substances. Elem Educ Online. 2021;20(5):4282–9.
18. Sharma A, Kumar SK. Review on identification and quantification of genotoxic impurities. Int J Health Sci. 2022;6(S7):4043–65.

HOW TO CITE: Vishakha Pawar, Pallavi Pawar, Akanksha Gangurde, Pankaj Aher, Mitesh Sonawane, Avish Maru, Analytical Research & Development on Genotoxic Impurities, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 10, 1279-1291. https://doi.org/10.5281/zenodo.17341882