A Comprehensive Review of Current and Emerging Analytical Techniques for the Identification, Quantification, and Assessment of Genotoxic Impurities in Drug Substances

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Abstract

Identifying and quantifying genotoxic impurities (GTIs) in drug substances at trace levels is a difficult task that necessitates using sophisticated, hyphenated analytical techniques. This study provides a complete overview of the current analytical methodologies used for the detection and measurement of GTIs in pharmacological compounds. It focuses on risk assessment and the many analytical approaches used by regulatory agencies and researchers. This review outlines the numerous sources of GTIs while also digging into the industrial processes that lead to their development. A comprehensive range of analytical techniques, including both chromatographic and non-chromatographic approaches, is thoroughly described. Popular analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry single quad LCMS, GCMS, and triple quad approaches have distinct applications, strengths, and limitations. Capillary electrophoresis (CE), LC-MS/MS, GC-MS/MS, LC-HRMS/MS, and Microbial reverse mutation assay (Ames) for analyzing genotoxic impurities, as well as other hyphenated techniques, were discussed. In addition, The review addresses the issues encountered in GTI analysis, including setting acceptance criteria, defining appropriate reference standards, and validating analytical methodologies. Regulatory rules and requirements established by governing organizations are also investigated. Furthermore, emerging trends and breakthroughs in the field, such as in-silico prediction tools, novel sample preparation processes, and rapid screening approaches, are highlighted. The use of quality-by-design (QbD) principles and automated technologies to improve efficiency is also highlighted. This evaluation is a significant resource for researchers, regulatory bodies, and pharmaceutical companies.

Keywords

Genotoxic impurities (GTIs), Regulatory guidelines, Drug substances, Analytical techniques, Safety, and quality assurance,

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1. Introduction

Genotoxic impurities (GTIs) in drug substances have garnered significant attention in the pharmaceutical industry due to their potential to damage genetic material [1-4]. In addition, pose risks to human health. The detection and quantification of these impurities is critical for ensuring the safety and quality of the product throughout their development and manufacturing processes meeting cGMP compliance [5]. The presence of even trace amounts of genotoxic impurities in drug formulations can possess significant risks to human safety and it is a most serious concern leads to product recall [6].
Over the years, significant advancements have been made in analytical techniques aimed at effectively detecting and measuring Genotoxic impurities (GTIs) and Potential genotoxic impurities (PGIs) are identified by structural alerts relationship. If a structure is not part of the cohort of concern, the existence of impurity structural warnings alone is not thought to be sufficient to trigger follow-up actions. The results of a bacterial mutagenicity assay should be predicted using (Q)SAR techniques in a computational toxicology evaluation. It is best to use two complementary (Q)SAR prediction approaches. Two approaches, one based on expert rules and the other on statistics, should be used. The Organisation for Economic Cooperation and Development (OECD) has produced wide validation requirements for (Q)SAR models utilizing multiple prediction methodologies.

Some structural groups have been proven to be so strong that intakes even below the TTC could theoretically be related to considerable cancer risk. This "cohort of concern" of highly potent mutagenesis carcinogens includes aflatoxin-like, N-nitroso, and alkyl-azoxy compounds.

**Table 1:** Here are some examples of structural alerts, and Toxic hazard data collected from Toxtree software

<table>
<thead>
<tr>
<th>Group</th>
<th>Structural alert</th>
<th>Toxic Hazard (Toxtree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic amines:</td>
<td><img src="image" alt="Aniline" /></td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Benzidine" /></td>
<td>High</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="4-aminobiphenyl" /></td>
<td>High</td>
</tr>
</tbody>
</table>
Epoxides are cyclic chemical compounds with a three-membered ring comprising two carbons and one oxygen. Epoxides have been shown to be genotoxic, causing DNA damage [9,10]. Epoxide-containing substances include ethylene oxide and propylene oxide.

Quinones: These aromatic compounds contain a double bond with an oxygen atom. Quinones are known to be toxic and can cause oxidative stress, [11,12]. Examples of compounds that contain quinones include naphthoquinone and anthraquinone.

Nitro groups: These substructures contain a nitrogen atom attached to two oxygen atoms. Nitrogen groups have been shown to be harmful [13,14]. This can result in methemoglobinemia, a condition in which the blood cannot carry oxygen. [15,16]. Examples of compounds that contain nitro groups include nitroglycerin and dinitrotoluene.
Halogenated compounds: These compounds contain one or more halogen atoms (e.g., fluorine, chlorine, bromine, or iodine). Compounds with halogens [17]. Are toxic and can induce a variety of negative effects, including organ damage and cancer. Examples of compounds that contain halogenated groups include chloroform, fluoroacetate, and bromobenzene.

Following the examination of structure with in-silico tools, the next stage is the quantification of possible genotoxic contaminants, for which regulatory bodies and researchers, employing a suitable analytical technique, create numerous analytical methods.

According to the FDA’s CGMP rules for nitrosamines [18]. To meet the low AIs advised for nitrosamines, sensitive techniques with limits of quantification (LOQ) in the parts-per-billion (ppb) range are frequently required. Nitrosamines with LOQs of less than 0.03 ppm. The detection and quantification limits, however, are determined by the analytical technique and detector response in relation to the analyte concentration.

**Table 2:** Limit of detection (LOD) and Limit of quantification (LOQ) for USFDA and ANSM methods

<table>
<thead>
<tr>
<th>Analytical Technique</th>
<th>LOD for the method</th>
<th>LOQ for method</th>
<th>Genotoxic impurity</th>
<th>Method Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC-UV</td>
<td>0.1ppm</td>
<td>0.3 ppm</td>
<td>NDMA in Valsartan</td>
<td>ANSM Method reference no 18A0399-02 [19].</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>0.01ppm</td>
<td>0.03 – 3.33 ppm</td>
<td>NDMA in Ranitidine method</td>
<td>US Food and Drug Administration (2019). LC-MS/MS method for determining NDMA in ranitidine drug substance and drug product [20].</td>
</tr>
<tr>
<td>LC-HRMS</td>
<td>0.01ppm</td>
<td>0.03 to 0.1ppm</td>
<td>NDMA in ARB Drugs</td>
<td>US FDA. - LC-HRMS NDMA detection method in metformin drug material and drug product. (2020). [21].</td>
</tr>
<tr>
<td>LC-ESI-HRMS</td>
<td>0.005ppm</td>
<td>0.01 to 0.1ppm</td>
<td>NDMA in Metformin</td>
<td>US FDA. LC-ESI-HRMS method for the determination of</td>
</tr>
</tbody>
</table>
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**Section A - Research paper**

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS/MS</td>
<td>0.005ppm</td>
<td>0.008ppm</td>
<td>NDMA in Valsartan</td>
<td>US FDA method for Impurity Assay by GC-MS/MS of Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA), (2019). [23].</td>
</tr>
<tr>
<td>GC-MS-HS</td>
<td>0.05ppm</td>
<td>0.3ppm</td>
<td>NDMA in Valsartan</td>
<td>US FDA. GC/MS Headspace Method for NDMA Detection in Valsartan Drug Substances and Drug Products. [24].</td>
</tr>
</tbody>
</table>

The LOD and LOQ values in the graph are derived from regulatory agencies' (USFDA and ANSM) published methods of analysis for the identification and quantification of NDMA impurity in drug substances.

**Figure 1:** Analytical Methods for NDMA impurity with LOD and LOQ

The LOQ 0.03ppm sensitive methods are most suited for NDMA impurity analysis, according to FDA CGMP requirements.
The best analytical procedures for genotoxic impurities like NDMA, as shown in Table 2 and the LOD and LOQ graph above, are GC-MS/MS, LC-ESI-HRMS, LC-HRMS, and LC-MS/MS.

Several researchers, in addition to regulatory bodies, offered analytical methods for genotoxic impurities. Chittireddy et al established a GC-MS/MS approach for the detection of alkyl halides' probable genotoxic impurities in posaconazole [25-30]. Matveeva et al [31] emphasize the importance of sensitive and specialized analytical procedures for detecting genotoxic substances in pharmaceutical goods at very low levels. Al Azzam KM et al examined methods for detecting genotoxic impurities in pharmaceuticals using HPLC, CE, and GC [32]. Other complementary approaches for small molecules are being researched and implemented [33-36].

This paper provides an overview of the current analytical techniques used for identifying and quantifying GTIs in drug substances, as well as an examination of the sources of GTIs, such as process-related impurities and degradation products, as well as the manufacturing procedures that can lead to their formation. Understanding the sources of GTIs is crucial for developing effective control strategies and reducing their presence in psychoactive substances. The review emphasizes chromatography and non-chromatography methods, which are the most often utilised approaches in GTI analysis. For the separation and detection of GTIs, hyphenating techniques such as the combining of Chromatography with mass spectroscopy techniques such as liquid chromatography-mass spectroscopy (LC-MS) and gas chromatography-mass spectroscopy (GC-MS) GTI separation and detection requires great sensitivity and selectivity. Non-chromatographic technologies, like as capillary electrophoresis (CE) and the Microbial reverse mutation assay (Ames), provide alternate GTI analysis procedures with distinct advantages in particular contexts.

To establish a comprehensive understanding of the strengths and limitations of each technique, this review thoroughly examines the specific applications of HPLC, GC, LC-MS, GC-MS, LC-MS/MS, GC-MS/MS, ICP-MS, CE, and other non-chromatographic techniques like Microbial reverse mutation assay (Ames) in genotoxic impurity analysis. It discusses the parameters and considerations involved in selecting the appropriate technique for a given analytical challenge; however, analyzing GTIs poses several challenges including the availability of genotoxic impurity standards, and analytical challenges, Liu, David Q, et al reported Analytical challenges in genotoxic impurity stability testing [37]. Analytical challenges include method selection, optimization, Matrix effect, selectivity, sensitivity, resolution among impurities, and API’s establishing limit of detection, limit of quantification in trace levels, repeatability, and reproducibility. Establishing acceptance criteria for GTIs, determining suitable reference standards, and validating analytical methods are critical steps in GTI analysis. Regulatory agencies play a vital role in setting guidelines and requirements for GTI control in drug substances. ICH M7 guideline outlines how to calculate theoretically acceptable amounts of human exposure for mutagenic contaminants in the absence of adequate experimental carcinogenicity data. The toxicological concern (TTC) level [38-40]. For example, is a commonly used permissible consumption level calculated from linear extrapolation of preclinical TD50 [41-45]. This review discusses the existing regulatory landscape, providing insights into the expectations and guidelines of regulatory authorities. Furthermore, this review highlights the sources of genotoxic impurities in drug substances. These impurities can originate from various stages of the drug manufacturing process, including starting materials, intermediates, catalysts, reagents, and degradation products [46]. Understanding the potential sources of genotoxic impurities is crucial for implementing effective control strategies and developing appropriate analytical methods. Emerging trends and advancements in the field of genotoxic impurity analysis. In-silico predictive tools [47-49]. Such as quantitative structure-activity relationship (QSAR) models and expert systems [50-53]. (QSAR) models have gained prominence in predicting and assessing the genotoxic potential of impurities.
Novel sample preparation techniques like derivatization methods [54-58]. And non-derivatization sample preparation methods [59,60]. Including solid-phase microextraction (SPME) [61,62]. And dispersive liquid-liquid microextraction (DLLME) [63]. Offer efficient and rapid sample preparation for GTI analysis. Additionally, rapid screening methods, such as immunoassays and biosensors, have shown promise in providing quick and cost-effective assessments of GTIs. The integration of quality-by-design (QbD) principles and the use of automated systems in GTI analysis are also discussed. QbD principles enable a systematic and proactive approach to understanding and controlling GTIs during the development and manufacturing processes [64]. Automation enhances efficiency, and reduces human error, Sun, Mingjiang et al used the Quality by Design (QbD) approach to develop a systematic method for analyzing dimethyl sulfate in pazopanib HCl (Votrient) [65]. Székely, Gy, et al [66]. Used Design of Experiments (DoE) as a strategy for developing LC-MS/MS methods [67]. Katerina Grigori et al. used Chemometrics to develop and validate an LC-MS/MS method [68]. This comprehensive review serves as a valuable resource for researchers, regulatory authorities, and pharmaceutical manufacturers involved in genotoxic impurity analysis. By providing an in-depth understanding of current analytical techniques, challenges, regulatory guidelines, and emerging trends, this review aids in the effective management of genotoxic impurities, ensuring the safety and quality of drug products.

2. Methods

Systematic and comprehensive review is performed to find the comprehensive grasp of the current and emerging analytical techniques used to identify and measure genotoxic impurities as well as the risks they present. It also makes an effort to highlight the limitations of the present methods for the identification, confirmation, and management of genotoxic impurities in pharmaceutical substances. PubMed, ScienceDirect, Scopus, Web of Science, and regulatory guidelines were just a few of the scientific databases that were exhaustively searched. The review focuses on analytical methods for the identification and quantification of GTI in drug substances and Data were taken from a sizable number of research and review journals. Were thoroughly investigated, and relevant information regarding analytical methods for GTI analysis was acquired. Key details of each analytical approach under our review included data on genotoxic impurities present and past practices and trends, needs for validation, and legal and regulatory requirements, The retrieved data were synthesised to offer an in-depth overview of existing analytical approaches for GTI identification and quantification in pharmacological compounds. The information was organized and presented in a coherent manner, highlighting the strengths and limitations of each method, regulatory considerations, challenges, and advancements in the field, the review provides a comprehensive analysis of the current analytical techniques for identifying and quantifying genotoxic impurities in drug substances, ensuring the inclusion of relevant information and insights from a wide range of scientific literature and regulatory guidelines.

3. Genotoxic impurities classification

Genotoxic impurities (GTIs) are classified based on their potential to cause genetic material damage to humans and animals, the classification of genotoxic impurities is important for assessing their risk and determining the appropriate strategies for their control and regulation. In addition, Jacobson-Kram, David, et al examined practical and theoretical strategies for qualifying several classes of impurities [69]. ICH M7 R1 provides guidelines and classifications for genotoxic impurities, these are four classes of genotoxic impurities Class-1 impurities are mutagenic and carcinogens; depending on the relevant animal studies and mechanistic understanding, these impurities are either strongly suspected to be human carcinogens or have sufficient evidence to establish their propensity to cause cancer in humans. Most at danger from these impurities is the health of people, and there is minimal proof that
Class 2 impurities pose a risk for human cancer because they are recognised mutagens with an uncertain level of carcinogenicity. Class 3 These may produce promising results in some animal experiments or they may behave in a genotoxic manner in the studies, and Class 3 impurities have an alerting structure and are unrelated to the structure of the drug substance. They must be controlled or minimized to levels below acceptable limits using the proper TTC approach or a bacterial mutagenicity assay; if they are not mutagenic, they fall under Class 5 or if resulting in Ames test positive should be classified as Class 2.

Class 4 impurities have alerting structures or compounds related to drug substances or intermediates that have been tested and are non-mutagenic to be treated as non-mutagenic impurities and Class 5 impurities have no structural alarms or enough data to show that it is not mutagenic or carcinogenic to be treated as non-mutagenic impurities, and are not genotoxic, posing a risk to human health. The International Conference on Harmonisation (ICH) guidelines provide specific guidelines and standards for the control of genotoxic impurities in pharmaceutical products. These genotoxic impurities must first be classified before risks can be assessed, acceptable limits can be established, and the best analytical techniques for their detection and quantification in pharmaceutical products can be determined.

4. Sources of Genotoxic impurities

Genotoxic impurities (GTIs) can originate from various sources throughout the drug development and manufacturing process [70]. Understanding the potential sources of genotoxic impurities is crucial for implementing effective control strategies and developing appropriate analytical methods. Here are some common sources of genotoxic impurities

4.1 Starting Materials:

The Sources of genotoxic impurities are raw materials, intermediates, reagents, solvents, and catalysts used in the synthesis of Active pharmaceutical ingredients (APIs). Alkyl halides are chemical compounds that are used as raw material and contain one or more halogen atoms, such as chlorine, bromine, fluorine, or iodine, and these halo-alkanes have high reactivity, convenience of use, and are cost-effective, and they are extensively used in alkylation processes as starting materials or reagents in
the synthesis of active pharmaceutical ingredients (APIs), and even trace levels of these chemicals can alkylate DNA [71].

4.2 By-products and Degradation Products:

During the synthesis or manufacturing process, chemical reactions can produce impurities as by-products or degradation products. These impurities can arise from side reactions, hydrolysis, oxidation, or other degradation pathways. Impurities from catalysts, solvents, or other process-related factors can also contribute to the formation of genotoxic impurities. Jamrógiwicz, Marzena, et al. reported that Ranitidine under photo exposition results in the production of volatile degradation products [72].

4.3 Residual Solvents:

Some solvents used in the manufacturing process can contain genotoxic impurities. Residual solvents, such as organic solvents or cleaning agents, may carry traces of impurities that have genotoxic potential. Example Ethylene Dichloride, Benzene (Class I residual solvents) as per ICH Q3C guidelines [73].

4.4 Impurities from Packaging and Storage:

Genotoxic impurities can also originate from the packaging materials or storage conditions. For instance, leaching of impurities from containers, closures, or packaging materials into the drug product can introduce genotoxic impurities.

4.5 The presence of genotoxic impurities in drug substances can be influenced by environmental conditions. Pollutants, pesticides, or other impurities from the air, water, or soil, for example, can find their way into the drug manufacturing process and contaminate the final product. Stiborová, Marie, et al investigated the mechanism of carcinogenicity of 2-methoxyaniline (o-anisidine), an industrial and environmental pollutant [74]. Mani, Sujata et al studied the effect of triphenylmethane dye used as in human and veterinary medicine as a biological stain and its toxic, genotoxic, and carcinogenic effects on the environment [75]. Hayden, Patrick J., et al conducted studies for genotoxic inhalable chemicals using comet assay on human tissue models [76]. Hayden, Patrick J., et al studied metal genotoxic impurities in Water for Injection (WFI) [77,78]. Masood, Farhana, et al proposed methods for genotoxicity testing of environmental pollutants [79]. Chmielińska, Katarzyna, et al studied the impact of cyclic mustard gas impurities on the environment [80]. Industrial activities, waste incineration, and other sources can lead to environmental contamination by pollutants like polycyclic aromatic hydrocarbons (PAHs) [81,82], dioxins, and persistent organic pollutants (POPs) [83,84]. If drug substances are exposed to these impurities during production or storage, there is a risk of introducing genotoxic impurities.

The Environmental Protection Agency (EPA) set health reference levels for NMBA (30 ng/l), NDEA (0.4 ng/l), NDMA (0.6 ng/l), NDPA (7ng/l), NMEA (3 ng/l), and NPYR (2 ng/l) (EPA, 2016)
It is important for pharmaceutical manufacturers to thoroughly assess and monitor potential sources of genotoxic impurities throughout the entire drug development and manufacturing process. Implementing appropriate quality control measures, including rigorous testing of key starting raw materials to finished products and risk assessment strategies [85]. Can help identify and mitigate the presence of genotoxic impurities, ensuring the safety and quality of pharmaceutical products. Regulatory guidelines, such as those provided by organizations like the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), provide guidance on the control and qualification of genotoxic impurities in drug substances.

5. Current Regulatory Guidelines for Genotoxic Impurities

Regulatory guidelines play a crucial role in providing standards and recommendations for the control and qualification of genotoxic impurities (GTIs) in pharmaceutical products. Here are some current regulatory guidelines that address the assessment and management of genotoxic impurities.

The ICH has published several guidelines for the control of genotoxic impurities, as well as guidance on genotoxicity evaluation and data interpretation for pharmaceuticals intended for human use. [86,87]

5.1 ICH M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk: This guideline is concerned with the evaluation and management of mutagenic impurities that may pose a risk of causing cancer. It provides a framework for evaluating the genotoxic potential of impurities and establishing acceptable limits [88-95].

5.2 ICH Q3A (R2): This guideline addresses impurities in new drug substances and includes considerations for genotoxic impurities. It provides guidance on the qualification and control of impurities, including those with genotoxic potential according to Identification and Qualification Decision Tree mentioned in the ICH Q3A guidelines [96].

5.3 United States Pharmacopeia (USP):

USP provides standards and monographs for pharmaceutical products. It includes specific chapters and guidelines related to genotoxic impurities, such as USP <1663> Assessment of Genotoxic Impurities in Pharmaceuticals: This chapter provides guidance on the assessment of genotoxic impurities in pharmaceutical products. It outlines testing strategies and acceptance criteria for evaluating the genotoxic potential of impurities [97-99].
5.4 European Medicines Agency (EMA):

EMA provides guidelines EMEA/CHMP/QWP/251344 [100], and regulatory requirements for the pharmaceutical industry within the European Union. Guidelines on residual solvents EMA/CHMP/ICH/82260/2006, and ICH M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, this guideline is an adaptation of the ICH M7 guideline and provides guidance for the assessment and control of mutagenic impurities with potential carcinogenic risk in pharmaceutical products and EMA/CHMP/CVMP/SWP/169430/2012 Guideline on the Limits of Genotoxic Impurities and provides recommendations on the setting of limits for genotoxic impurities in pharmaceutical products. It includes information on the assessment, qualification, and control of genotoxic impurities, It is important for pharmaceutical manufacturers to follow these regulatory guidelines and incorporate them into their drug development, manufacturing, and quality control processes to ensure compliance and the safety of pharmaceutical products. It is also essential to stay updated with the latest revisions and updates to these guidelines, as regulatory requirements may evolve over time, In a referral under Article 31 of Directive 2001/83/EC, procedure EMEA/H/A-31/1471, the risks connected to the presence of the nitrosamines N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in sartan blood pressure medications (angiotensin II receptor blockers) containing a tetrazole ring have been evaluated. Acceptable intakes (AI) of 96.0 ng for NDMA and 26.5 ng for NDEA have been established limits based on the TD50 values in rat carcinogenicity studies [101,102].

The Carcinogenic Potency Database (CPDB, 2007), which provides information on animal carcinogenicity, is the most complete source. A mathematical model was used to determine the dose (TD50) that causes cancer in 50% of the animals in this database's 6540 long-term animal cancer studies involving 1547 substances. Table 3, lists the TD50 values from the CPDB for the N-nitrosamines described in this report, arranged by their descending carcinogenic potency (harmonic mean TD50).

**Table 3:** TD50 values for various N-nitrosamines discovered in the CPDB reported as per EMA Assessment Report EMA/369136/2020, [103].

<table>
<thead>
<tr>
<th>Name of the Chemical</th>
<th>Abbreviation</th>
<th>TD50 [mg/kg/ day] harmonic mean rat, CPDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroso-N-methyl-N-(2-phenyl)ethylamine</td>
<td>NMPEA</td>
<td>0.00998</td>
</tr>
<tr>
<td>N-Nitrosodiethylamine</td>
<td>NDEA</td>
<td>0.026</td>
</tr>
<tr>
<td>N-Nitrosomethylethylamine</td>
<td>NMEA</td>
<td>0.053</td>
</tr>
<tr>
<td>N-Nitrosodimethylamine</td>
<td>NDMA</td>
<td>0.096</td>
</tr>
<tr>
<td>N-Nitrosonornicotine</td>
<td>NNN</td>
<td>0.096</td>
</tr>
<tr>
<td>4-(N-Nitrosomethylamino) -1-(3-pyridyl)-1- butanone</td>
<td>NNK</td>
<td>0.0999</td>
</tr>
<tr>
<td>N-Nitrosomorpholine</td>
<td>NMOR</td>
<td>0.109</td>
</tr>
<tr>
<td>N-nitrosomethylaniline</td>
<td>NMPA</td>
<td>0.142</td>
</tr>
</tbody>
</table>
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Section A - Research paper

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>TD 50 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Nitrosodi-n-propylamine</td>
<td>NDPA 0.186</td>
</tr>
<tr>
<td>Nitrosodibutylamine</td>
<td>NDBA 0.691</td>
</tr>
<tr>
<td>N-nitrosopyrrolidine</td>
<td>NPYR 0.799</td>
</tr>
<tr>
<td>N-Methyl-N’-nitro-N-nitrosoguanidin</td>
<td>MNNG 0.803</td>
</tr>
<tr>
<td>N-Methyl-N’-nitro-N-nitrosoguanidin</td>
<td>NMBA 0.982</td>
</tr>
<tr>
<td>N-Methyl-N’-nitro-N-nitrosoguanidin</td>
<td>NPIP 1.43</td>
</tr>
<tr>
<td>N-Nitrosodiethanolamine</td>
<td>NDELA 3.17</td>
</tr>
<tr>
<td>N,N-diisopropylethyl-N-ethylamine</td>
<td>DIPNA 0</td>
</tr>
<tr>
<td>N-nitrosodiphenylamine</td>
<td>NDPhA 167</td>
</tr>
</tbody>
</table>

Figure 4: The TD50 [mg/kg/day] value was obtained from the harmonic mean of rats, data taken from Carcinogenic Potency Database (CPDB).

From the Table 3 data and graph, NMPEA is having the lowest value 0.00998 mg/Kg/day.

Table 4: The Carcinogenic Potency Database (CPDB) provides the TD50 (mg/kg/day) values of the most potent chemicals collected from rat studies.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroso-1,2,3,6-tetrahydropyridine</td>
<td>0.0601</td>
</tr>
<tr>
<td>N-Nitroso-2,3-dihydroxypropyl-2-hydroxypropylamine</td>
<td>0.0535</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>0.053</td>
</tr>
<tr>
<td>Nitrosoethylmethylamine</td>
<td>0.0503</td>
</tr>
<tr>
<td>Azoxymethane</td>
<td>0.0466</td>
</tr>
<tr>
<td>N-Nitrosomethyl-2-hydroxypropylamine</td>
<td>0.0463</td>
</tr>
<tr>
<td>Nitrosoheptamethyleneimine</td>
<td>0.0378</td>
</tr>
<tr>
<td>Chlorozotocin</td>
<td>0.0375</td>
</tr>
<tr>
<td>Nitroso-2,3-dihydroxypropyl-2-oxo-propylamine</td>
<td>0.0352</td>
</tr>
<tr>
<td>Hexamethylphosphoramide</td>
<td>0.0344</td>
</tr>
<tr>
<td>N-Nitrosodiethylamine</td>
<td>0.0265</td>
</tr>
<tr>
<td>Z-Ethyl-ON,N-azoxylane</td>
<td>0.022</td>
</tr>
<tr>
<td>Cadmium sulphate (1:1)</td>
<td>0.0217</td>
</tr>
<tr>
<td>Z-Ethyl-ON,N-azoxymethane</td>
<td>0.0189</td>
</tr>
<tr>
<td>N-Nitrosomethyl(2-oxopropyl) amine</td>
<td>0.0172</td>
</tr>
<tr>
<td>Aristolochic acid, sodium salt</td>
<td>0.0141</td>
</tr>
<tr>
<td>Cadmium chloride</td>
<td>0.0136</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>0.0114</td>
</tr>
<tr>
<td>Nitroso-N-methyl-N-(2-phenyl) ethylamine</td>
<td>0.00998</td>
</tr>
<tr>
<td>Trenimon</td>
<td>0.00504</td>
</tr>
<tr>
<td>Bis-(chloromethyl)ether</td>
<td>0.00357</td>
</tr>
<tr>
<td>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.0032</td>
</tr>
<tr>
<td>Aflatoxin, crude</td>
<td>0.00299</td>
</tr>
<tr>
<td>2-Azoxo propane</td>
<td>0.00268</td>
</tr>
<tr>
<td>Aflatoxicol</td>
<td>0.00247</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>0.00111</td>
</tr>
<tr>
<td>Mitomycin-C</td>
<td>0.00102</td>
</tr>
<tr>
<td>HCDD mixture</td>
<td>0.000596</td>
</tr>
<tr>
<td>1-Azoxo propane</td>
<td>0.000241</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzo-p-dioxin</td>
<td>0.0000235</td>
</tr>
</tbody>
</table>
A Comprehensive Review of Current and Emerging Analytical Techniques for the Identification, Quantification, and Assessment of Genotoxic Impurities in Drug Substances

Section A

5.5 FDA Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products, Recommended Approaches, FDA guidance document provides general recommendations for the identification, qualification, and control of genotoxic and carcinogenic impurities in drug substances and products. It offers guidance on assessing impurities with potential genotoxicity and carcinogenicity [104].

On 28 February 2019, FDA updated a table of interim acceptable intake limits for nitrosamine impurities to reflect N-Nitroso-N-methyl-4-aminobutyric acid (NMBA) limits, which are the same as those for NDMA. If laboratory testing confirms the presence of nitrosamine impurities in finished drug products, the agency will use the interim limits below to recommend manufacturers conduct a voluntary recall. The FDA is collaborating with industry and international agencies to guarantee that no contaminants access the market. However, we are tolerating the impurities below the level established in the table for a short period of time to avoid a possible shortage of ARBs.

FDA revised interim limits for nitrosamine impurity in ARBs in February 2019 shown in Table 4.

**Table 5:** Interim Acceptable Intake (AI) Limits for NDMA, NDEA, and NMBA in Angiotensin II Receptor Blockers (ARBs) [105].

<table>
<thead>
<tr>
<th>Name of the Drug</th>
<th>Max Daily Dose (mg/day)</th>
<th>(AI) NDMA (ng/day)*</th>
<th>(AI) NDMA (ppm)**</th>
<th>(AI) NDEA (ng/day)*</th>
<th>(AI) NDEA (ppm)**</th>
<th>(AI) NMBA (ng/day)*</th>
<th>(AI) NMBA (ppm)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azilsartan</td>
<td>80</td>
<td>96</td>
<td>1.2</td>
<td>26.5</td>
<td>0.33</td>
<td>96</td>
<td>1.2</td>
</tr>
<tr>
<td>Candesartan</td>
<td>32</td>
<td>96</td>
<td>3</td>
<td>26.5</td>
<td>0.83</td>
<td>96</td>
<td>3</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>800</td>
<td>96</td>
<td>0.12</td>
<td>26.5</td>
<td>0.033</td>
<td>96</td>
<td>0.12</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>300</td>
<td>96</td>
<td>0.32</td>
<td>26.5</td>
<td>0.088</td>
<td>96</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Figure 5:** The TD50 [mg/kg/day] value was obtained from the harmonic mean of rats, data of most potent chemicals taken from the Carcinogenic Potency Database (CPDB).

Figure 5: The TD50 [mg/kg/day] value was obtained from the harmonic mean of rats, data of most potent chemicals taken from the Carcinogenic Potency Database (CPDB).
A Comprehensive Review of Current and Emerging Analytical Techniques for the Identification, Quantification, and Assessment of Genotoxic Impurities in Drug Substances

Section A - Research paper

<table>
<thead>
<tr>
<th>Product</th>
<th>Method</th>
<th>Agency</th>
<th>GTI’S</th>
<th>Method LOQ Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>valsartan, losartan, irbesartan, olmesartan, candesartan</td>
<td>GC-MS/MS</td>
<td>Swissmedic OMCL</td>
<td>NDMA, NDEA, EIPNA, DIPNA, DPNA, DBNA</td>
<td>LOQ 15 ppb</td>
</tr>
<tr>
<td>Ranitidine Drug Substance and Film Coated Tablets</td>
<td>LC-MS/MS</td>
<td>German OMCL at the Chemisches und Veterinär-Untersuchungsamt (CVUA)</td>
<td>NDMA</td>
<td>sample solution (0.5 ng/ml – 30 ng/ml) in drug substance and film-coated tablets (0.05 ppm – 3 ppm)</td>
</tr>
</tbody>
</table>

The interim table shows the Acceptable intake of NDMA and NMBA in ng/day is 96 ng/day
* The allowable intake is daily exposure to a chemical such as NDMA, NDEA, or NMBA that has a cancer risk of one in 100,000 after 70 years of exposure.
** These figures are based on a drug’s maximum daily dose as stated on the label.
*** For the time being, the FDA is not objecting to losartan with NMBA levels less than 9.82 ppm remaining on the market.

Three more genotoxic impurities were added by FDA in 2021 to the existing list of nitrosamines, these are nitrosomethylphenylamin (NMPA), N-nitrosoisopropylethyl amine (NIPEA), and N-nitrosodiisopropylamine (NDIPA), and also provided guidance to the industry in revision-1 for the control of nitrosamine impurities found in human drugs. Acceptable intake limits for NDMA are 96 ng/day, NDEA is 26.5 ng/day, NMBA is 96 ng/day, NMPA is 26.5 ng/day, NIPEA is 26.5 ng/day, and NDIPA is 26.5 ng/day, along with its formation, structure, root cause [106].

The Nitrosamines International Strategic Group (NISG) was established in 2018 by a group of regulatory authorities in response to incidents involving nitrosamines around the world. This group shares information through multi-lateral teleconferences and external communications, contextualizing the risk to public health, scientific knowledge about the sources of contamination, and analytical techniques used to test potency. Another subgroup called the "Nitrosamines International Technical Working Group" (NITWG) was created in the year 2020 in order to share scientific information and current theories on technical safety and quality issues pertaining to nitrosamines and, where possible to promote technical convergence among member nations. [107].

6. Current analytical techniques employed for genotoxic impurities quantification in drug substances

Current analytical techniques for detecting and quantifying genotoxic impurities (GTIs) in drug substances employ various techniques to ensure the safety and quality of pharmaceutical products.

Apart from the US-FDA, the Council of Europe and edqm provides some publically available Analytical methods published by various regulatory agencies like Swiss OMCL, OMCL-BW Germany, ANSM French-OMCL method.

Table 6: Some of the analytical methods by various health regulatory agencies

<table>
<thead>
<tr>
<th>Product</th>
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<th>Agency</th>
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<td>NDMA</td>
<td>sample solution (0.5 ng/ml – 30 ng/ml) in drug substance and film-coated tablets (0.05 ppm – 3 ppm)</td>
</tr>
</tbody>
</table>
6.1 High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) are frequently used chromatographic techniques for determining impurities in drug substances. Based on their retention periods and peak regions, impurities are separated, recognized, and quantified using a chromatographic column, mobile phase, and detector. Specialized HPLC variations, such as reverse-phase HPLC, normal-phase HPLC, or ion exchange HPLC, are also utilized. UV, photodiode array (PDA) detectors for UV active substances, and IR detectors are among the detector types that are frequently used. Jenny Wang et al. developed an HPLC method to look for the genotoxic impurity hydrazine in pharmaceuticals. [108]. Jain, Mohit, et al developed a five potential genotoxic impurity method using HPLC with a UV detector in HILIC (Hydrophilic Interaction Liquid Chromatography) mode [109-110]. Fluorescence detectors for high sensitivity and specificity, refractive index detectors for UV inactive substances Using a photochemically induced fluorescence detector and HPLC, Michal Doua, et al. were able to identify genotoxic impurities in the vortioxetine manufacturing process. [111].
6.2 Gas chromatography (GC) is frequently employed for the identification of genotoxic substances that are volatile and semi-volatile. Using a chromatographic column and a gaseous mobile phase, impurities are separated. Dianne L. Poster et al. [112]. Cover the methods for identifying PAHs in environmental samples. The detection sensitivity and specificity are increased when gas chromatography is paired with mass spectrometry (MS) or an electron capture detector (ECD). For the identification and measurement of genotoxic impurities in the modern era, mass spectrometry (MS) is a potent analytical technique. It involves ionizing and fragmenting the impurities, followed by mass analysis to determine their molecular weights and structural information [113]. And combining chromatographic and spectral methods in hyphenated techniques [114].

6.3 For genotoxic impurity limit measurement, the combination of liquid chromatography (LC) and mass spectrometry (MS) is the most often employed hyphenated approach. While MS is a detection method that offers information about the molecular weight and structure of the analytes, LC is a separation technique that enables the separation of complicated mixtures into separate components, allowing for the utilization of both methods' advantages like HPLC and mass spectrometry may be coupled (LC-MS) [115]. Or GC (GC-MS) gas chromatography coupled with mass spectrometry [116]. To provide a comprehensive analysis of genotoxic impurities, charged species, including impurities that are genotoxic, are separated using capillary electrophoresis (CE), which separates them based on their electrophoretic mobility. It provides excellent resolution and sensitivity for impurity analysis [117,118].

Ames Assay or Ames test [119-123]. Are used to assess the mutagenic potential of genotoxic impurities. These tests utilize bacterial strains like salmonella [124-127]. With mutations in their DNA repair mechanisms to detect the presence of mutagenic compounds [128]. Sasaki, Yu F., et al. done a comparison of comet assay results and carcinogenicity [129].

6.4 In-Silico Tools are Computational tools such as (Quantitative) Structure-Activity Relationship ([Q] SAR) models with database [130-135]. For already identified genotoxic impurities, like Toxicity Estimation Software Tool (TEST) developed by the United States Environmental Protection Agency US-EPA [136]. Computer-assisted evaluation of industrial chemical substances according to regulation (CAESAR) [137]. Organization for Economic Co-operation and Development (OECD) provided a Guidance document on the validation of (Q) SAR models [138]. In addition, other similar computational tools are Toxtree, EPI Suite, Lazar OECD QSAR Application Toolbox, OncoLogic, PASS, and other commercially available software tools are ACD/Tox Suite, ADMET Predictor, BioEpisteme, Derek, Hazard Expert, MDL QSAR, Molcode Toolbox, MultiCASE, OASIS TIMES, TOPKAT, ToxAlert, q-Tox, CSGenoTox these models are used to predict the genotoxic potential of impurities based on their chemical structures. These tools can provide initial screening and assessment before experimental testing [139-144].

It is important for pharmaceutical manufacturers to select appropriate analytical methods based on the specific characteristics of the genotoxic impurities and the requirements of regulatory guidelines. And the validation of these tools is important for the right prediction, Contrera et al validated Toxtree and SciQSAR using a publicly available benchmark mutagenicity database and also assessed their applicability for the qualification of impurities in pharmaceuticals [145]. In addition, the developed methods should be validated as per ICH guidelines before implementation, to ensure accurate and reliable detection and quantification of genotoxic impurities in drug substances.

6.5 The LC-MS/MS (Liquid Chromatography-Mass Spectrometry) triple quad and GC-MS/MS (Gas Chromatography-Mass Spectrometry) triple quad are the two commonly used combination analytical
techniques for the detection and quantification of organic genotoxic impurities (GTIs) in drug substances.

To identify, isolate, and quantify genotoxic impurities in drug substances, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used, where Impurities are separated using a liquid chromatographic column, and then detected using mass spectrometry. [146-149]. Manchuri, Krishna Moorthy, and colleagues developed a UHPLC-MS/MS method for identifying and quantifying Bis (2-Chloroethyl) Amine, a genotoxic impurity in aripiprazole [150].

Chen Yuyuan et al developed 6 Potential genotoxic impurity methods in 5-difluoromethoxy-2-mercapto-1H-benzimidazole which is a starting material for Pantoprazole sodium (PPZS) [151]. Multiple reaction monitoring (MRM) mode in tandem mass spectrometry improves the specificity and sensitivity by tracking particular mass-to-charge (m/z) transitions for target analytes. LC-MS/MS offers high sensitivity and selectivity, allowing for the detection and quantification of genotoxic impurities at low levels in complex matrices. Liquid chromatography and tandem mass spectrometry were utilized by Guo, Tian, et al. to quickly and simultaneously identify sulfonate ester genotoxic impurities in medicinal compounds. [152]. Three potential genotoxic impurities in rabeprazole formulations were quickly analyzed using LC-MS by Yenugu, Veera Manohara Reddy, et al. [153]. Four potential genotoxic impurities in the active pharmaceutical ingredients in TSD-1 were determined using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method established by Wang, Taiyu, et al. [154]. Li, Shuhong, et al. developed a UPLC-MS/MS method for Simultaneous and trace-level quantification of two potential genotoxic impurities in valsartan drug substance [155].

6.6 Gas chromatography and tandem mass spectrometry are combined analytical techniques known as GC-MS/MS (Gas Chromatography-Mass Spectrometry/Mass Spectrometry) to analyze genotoxic impurities. It entails the use of a gas chromatographic column to separate volatile or semi-volatile impurities, followed by mass spectrometry for detection and identification. Hari Naga Prasada Reddy, Chittireddy, et al. developed a GC-MS/MS method for allyl chloride, a possible genotoxic contaminant in Gemfibrozil. [156-163]. GC-MS/MS provides excellent sensitivity and selectivity for the analysis of volatile and thermally stable genotoxic impurities, multiple reaction monitoring (MRM) or selected reaction monitoring (SRM) modes in tandem mass spectrometry enable the targeted detection of specific analytes, Ahirrao, Vinod K., et al. developed a Time-dependent selected reaction monitoring (t-SRM)-based gas chromatography-tandem mass spectrometry method (GC-MS/MS) for trace level determination of genotoxic impurities in Alalevonadifloxacin mesylate [164].

6.7 LC-HRMS/MS (Liquid Chromatography High-resolution mass spectrometry) is an analytical technique that combines the separation capabilities of liquid chromatography (LC) with the high-resolution mass analysis provided by mass spectrometry (HRMS). LC-HRMS instrumentation typically consists of a liquid chromatography system, such as high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography (UPLC), coupled with a high-resolution mass spectrometer, such as a quadrupole time-of-flight (Q-TOF) or Orbitrap mass spectrometer used for rapid screening of genotoxic impurities. The data obtained from LC-HRMS analysis is typically processed and analyzed using specialized software to identify and quantify the compounds of interest, several LC-HRMS methods are published by US-FDA to identify and quantify NDMA (N-nitroso dimethylamine) in metformin and Ranitidine drugs, shown in Table 2, and other researchers reported several LC-HRMS methods for quantification of genotoxic impurities [165-168].

6.8 LC-GC-MS is a combination of Liquid Chromatography-Gas Chromatography-Mass Spectrometry analytical techniques to utilize the maximum capabilities of liquid chromatography, gas chromatography, and the power of mass spectrometry. [169-171].

6.9 LC-ICP-MS (Liquid chromatography-inductively coupled plasma-mass spectrometry), This technique uses inductively coupled plasma-mass spectrometry and liquid chromatography to detect and measure impurities that contain metals or other important elements. [172-176].

6.10 LC-NMR (Liquid Chromatography-Nuclear Magnetic Resonance) is an emerging and powerful analytical technique that combines liquid chromatography with nuclear magnetic resonance spectroscopy to identify and characterize impurities based on their structural properties [177-181]. The ability to identify, quantify, and structurally clarify genotoxic impurities in pharmaceutical compounds is improved because of the combination of analytical approaches. The optimum process is dependent on the type of impurities, the required sensitivity and selectivity, and the regulatory requirements that must be met. These approaches need to be confirmed in order to generate accurate and reliable results from genotoxic impurity analysis, additionally, as an enhancement to results integration software, precise computational methods are needed to forecast the structure of genotoxic impurities [182,183].

7. Current Control Strategies and Risk Assessment

Control measures for genotoxic impurities (GTIs) aim to limit their presence in pharmaceutical items in order to safeguard patients. Müller, Lutz, et al. [184] provide a method for testing, categorising, qualifying, assessing the toxicological risk of, and regulating contaminants with the potential to cause genotoxicity in pharmaceutical goods. Chris Barber and colleagues suggested a framework for guiding the adoption of ICH M7 control techniques. [185]. In their paper, Risk Assessment of genotoxic impurities for novel chemical entities, Teasdale, Andrew, et al. discussed the usefulness of employing an in silico evaluation technique. [186]. The ICH M7 Guideline emphasises Preventive Measures, Analytical Testing, and Risk Assessment Procedures for DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. [187-191].

7.1 Risk Assessment and Identification:

A broad decision tree was drawn based on standard industry practices and regulatory guidelines for identifying, measuring, and analyzing genotoxic impurities. its starts with risk assessment and ends with risk communication and control strategy which reflects standard methods and concerns used in
the pharmaceutical industry throughout drug development to assure product safety.

**Figure 6:** Decision tree Drawn based on standard industry practices and regulatory guidelines for identifying, measuring, and analyzing genotoxic impurities.

Identify potential sources of genotoxic impurities in the drug substances and products by conducting a complete risk assessment in accordance with the regulations in effect. [192]. Using the point of departure matrices, MacGregor et al. reported on the International Workshops on Genotoxicity Testing (IWGT) report on a quantitative method for genotoxicity risk assessment. [193]. G. E. Johnson et al. [194] described the Derivation of point of departure (PoD) estimates in genetic toxicology investigations and potential applications in risk assessment. Snodin, David J., et al. [195], published a critical analysis concentrating on N-nitrosamines, a mutagenic contaminant in pharmaceuticals, the cohort of concern, with an emphasis on N-nitrosamines. Humfrey, Charles DN et al. highlighted recent developments in risk evaluation of possibly genotoxic impurities in pharmaceutical medicinal compounds [196]. The structural alarms related to frequently occurring probable genotoxic impurities are examined by Reddy, Ambavaram Vijaya Bhaskar, et al. They also explore draft guidelines provided by various regulatory authorities to restrict the quantities of impurities in medicinal compounds and determine their toxicity. [197]. Identifying and regulating genotoxic impurities in the early stages of chemical process development for pharmacological substances, Duane A. Pierson et al explored the numerous sources of anticipated impurities in the synthesis of a drug substance. [198]. The amount of DNA adducts produced endogenously by regular cellular metabolism, oxidative stress, and everyday background exposures must all be taken into account when using DNA adducts to assist quantitative risk assessment. [199-204]. Using in-silico techniques to assess the genotoxic potential of impurities, based on their chemical structure, such as structure-activity relationship (SAR) models, To
determine whether genotoxic impurities may be present, take into account the impurity profiles of starting materials, intermediates, and process-related impurities.

7.2 Process Optimization and Design:
Implement quality by design (QbD), Kowtharapu, Leela Prasad, et al used Box-Behnken Design (BBD) to optimize the final method conditions [205]. and robust process optimization to minimize the formation of genotoxic impurities during drug synthesis or manufacturing. Utilize appropriate manufacturing techniques, such as closed systems or containment measures, to prevent cross-contamination and limit exposure to potential sources of genotoxic impurities.

7.3 Qualification and Control:
Develop and validate sensitive analytical methods on suitable instruments like LCMS, GCMS, ICP-MS, LC-MS/MS, GC-MS/MS, and LC-HRMS for the detection and quantification of genotoxic impurities. Set appropriate acceptance criteria and specifications for genotoxic impurities based on regulatory guidelines, risk assessment, and safety considerations. Implement regular testing of raw materials, intermediates, and final products to ensure compliance with specified limits for genotoxic impurities. Establish robust quality control systems to monitor and manage genotoxic impurities throughout the manufacturing process.

Packaging and Storage Considerations: Select appropriate packaging materials that minimize the risk of leaching or contamination by genotoxic impurities. Implement proper storage conditions to maintain the stability and integrity of the product and prevent the formation or introduction of impurities.

7.4 Regulatory Compliance and Documentation:
Follow regulatory guidelines for the control and qualification of genotoxic impurities, such as those published by the International Council for Harmonisation (ICH) and local regulatory agencies. Maintain thorough documentation and records of risk assessments, analytical methodologies, testing findings, and genotoxic impurity control strategies. Keep up to current on the newest regulatory standards and guidelines for genotoxic impurities, and adjust your control measures accordingly. Implementing these genotoxic impurity management measures helps to ensure that pharmaceutical products meet high-quality standards while minimizing potential threats to patient safety. Pharmaceutical producers must include these methods in their quality management systems while also adhering to appropriate regulatory standards and criteria.

8. Discussion
By summarizing the current state-of-the-art techniques, this review offers a comprehensive overview of the analytical strategies employed for the detection and quantification of GTIs, emphasizing their importance in drug substance risk assessment. And review highlights several key points as follows,

8.1 Advancements in Analytical Techniques:
This review focuses on various analytical techniques used for GTI analysis, including high-performance liquid chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC), gas chromatography (GC), mass spectrometry (MS), and capillary electrophoresis (CE). It analyses the benefits and drawbacks of each technique and emphasizes its use in diverse settings. Furthermore, it emphasizes the expanding use of combination techniques for GTI analysis, including LC-MS/MS, GC-MS/MS, and ICP-MS, which provide improved sensitivity, selectivity, and structure identification capabilities.
8.2 Importance of Sensitivity and Selectivity:
When dealing with GTIs, the discussion emphasises the vital importance of sensitive and selective analytical methodologies. The capacity to detect and quantify these contaminants at low levels is critical for patient safety, as even trace concentrations of GTIs can be harmful. To acquire accurate and dependable results in GTI analysis, the review goes into the importance of method validation, defining proper acceptance criteria, and implementing solid quality control processes.

8.3 Risk Assessment and Regulatory Guidelines:
The risk assessment is the key control step of GTIs' elimination or minimizing of the risk level in compliance with regulatory guidelines. The risk assessment outlines the importance of evaluating potential sources of GTIs, considering toxicological properties, and estimating safe exposure limits. The review also explores the current regulatory guidelines provided by regulatory authorities, such as the FDA, ICH, and other local agencies and their impact on the analytical strategies employed for GTI identification and quantification.

8.4 Challenges and Future Perspectives:
The discussion highlights the difficulties encountered in GTI analysis, such as the complexity of impurity profiles, the scarcity of reference standards, and the need for continuous method improvements. It also identifies topics for future research and development, such as the investigation of alternative methodologies, the development of better in silico tools for forecasting genotoxic potential, and the creation of more complete and harmonized regulatory requirements.

The European guideline adopts a Threshold of Toxicological Concern (TTC) approach, which utilizes animal carcinogenicity data and conservative assumptions to estimate a daily dose (1.5 μg/day) associated with a lifetime cancer risk of 1 in 100,000. This risk level is deemed acceptable for genotoxic impurities in human medicines. However, presenting the TTC as a single precise figure may imply an unwarranted level of accuracy. Hence, it is suggested that regulatory authorities, allowing a range within fivefold of the TTC limit, adopt a more flexible approach. The acceptance of this staged TTC approach has varied among regulatory authorities, leading to discrepancies in the evaluation of new drug products. Therefore, it is vital to establish a common agreement between the pharmaceutical industry and regulatory authorities worldwide. This agreement would ensure the development and timely delivery of new medicines while maintaining patient safety.

Overall, this assessment of existing and new analytical approaches for identifying and quantifying GTIs in pharmacological compounds gives a thorough grasp of the field's advances, obstacles, and future directions. It is a helpful resource for academics, pharmaceutical makers, and regulatory authorities in creating effective control techniques and maintaining pharmaceutical product safety and quality by regulating GTIs.

9. Conclusion
Identifying and quantifying genotoxic impurities (GTIs) in drug substances by using the latest hyphenated analytical techniques and in-silico tools can provide a comprehensive overview of the genotoxic impurities and helps to control these genotoxic impurities in the drug developmental stage to the manufacturing stage, starting from raw materials to finished product. The review highlights the importance of hyphenated techniques and their capabilities, accurate and reliable detection and quantification of GTIs to ensure the safety and quality of drug products. The discussion of various analytical techniques, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), and capillary electrophoresis (CE), showcases the diverse approaches...
employed for GTI analysis. The review emphasizes the increasing use of hyphenated techniques such as LC-MS/MS and GC-MS/MS, ICP-MS/MS, which offer enhanced sensitivity, selectivity, and structural identification capabilities. The review underscores the significance of sensitivity and selectivity in GTI analysis, as even trace amounts of GTIs can have detrimental effects on patient health. It emphasizes the need for method validation, appropriate acceptance criteria, and robust quality control systems to ensure accurate and reliable results.

Furthermore, the review highlights the importance of risk assessment in managing GTIs. It discusses the evaluation of potential sources, consideration of toxicological properties, and estimation of safe exposure limits. The review also addresses the impact of regulatory guidelines provided by authorities such as the FDA and ICH on the analytical strategies employed for GTI identification and quantification, despite the advancements, challenges persist in GTI analysis, including impurity profile complexity and limited availability of reference standards. The review identifies areas for future research, such as the exploration of alternative techniques, improvement of predictive tools, and harmonization of regulatory guidelines, this review serves as a valuable resource for researchers, pharmaceutical manufacturers, and regulatory authorities, providing insights into the current state-of-the-art analytical methods for GTI identification and quantification. By implementing these methods and adhering to regulatory guidelines, the pharmaceutical industry can continue to ensure the safety and quality of drug substances by effectively managing genotoxic impurities.

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Section A - Research paper


Matthias Bicker, Michael Müller, Marc Mittermüller, Daniel Haines and Uwe Rothhaar discuss
the regulatory requirements that need to be considered when designing an extractables and leachables study for a drug product or medical device. To illustrate the subject further, the authors provide two example studies, each following a different set of....." ISO 10993 (2021): 10993-18. https://ondrugdelivery.com/comparative-extractable-studies-for-injectables-and-medical-devices-aligned-with-usp-and-iso-10993-guidelines/


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