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

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

Srinivas.B[1,3], Bhaskar Mangalapu[1,2], Ramesha Andagar Ramakrishna[2]*, Madhusudana Reddy MB[1], Swagata Halder[1]*, P Venkata Narayana[1]

[1]Department of Chemistry, School of Applied Sciences REVA University, Yelahanka, Bangalore, India; [2]Flow Chem Pharma Pvt. Ltd., C41, KHB Industrial Area, Yelahanka New Town, Bangalore, India; [3]Trroy Life Sciences Pvt Ltd., Yelahanka Industrial Area, Bangalore, India, Pin-560064.

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)s 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

*Corresponding Authors Dr. Ramesha Andagar Ramakrishna, Flowchem Pharma Pvt Ltd, C41, KHB Industrial Area, Yelahanka New Town, Bangalore-560106, E-mail: dr.ramesha@flowchempharma.com, Dr. Swagata Halder Department of Chemistry, School of Applied Sciences, REVA University, Bangalore, E-mail: swagata.halder@reva.edu.in.
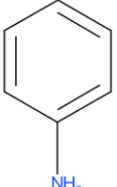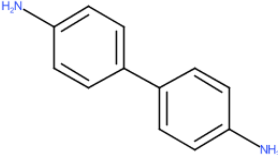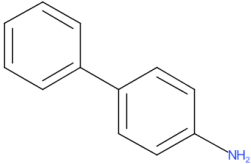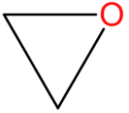
1. Introduction
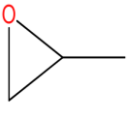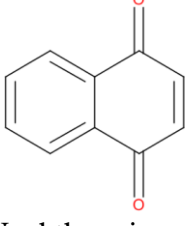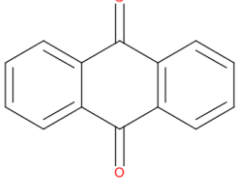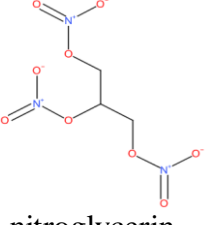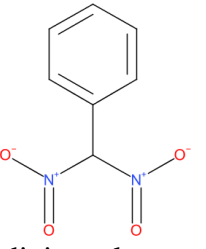
Genotoxic impurities (GTIs) in drug substances have garnered significant attention in the pharmaceutical industry due to their potential to damage genetic material [1-4]. In addition, pose risks to human health. The detection and quantification of these impurities is critical for ensuring the safety 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].

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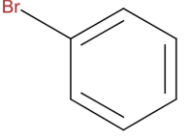
Over the years, significant advancements have been made in analytical techniques aimed at effectively detecting and measuring Genotoxic impurities (GTIs) and Potential genotoxic impurities (PGIs) are identified by structural alerts relationship, If a structure is not part of the cohort of concern, the 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 expe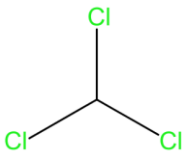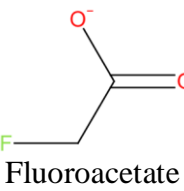rt rules and the other on statistics, should be used. The Organisation for Economic Cooperation and Development (OECD) has produced wide validation requirements for (Q)SAR models utilizing multiple prediction methodologies.

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

Group	Structural alert	Toxic Hazard (Toxtree)
Aromatic amines: These substructures contain an amino group attached to an aromatic ring. Aromatic amines are known to be mutagenic and can cause cancer [7,8]. Examples of compounds that contain aromatic amines include aniline, benzidine, and 4-aminobiphenyl.	Aniline	Low
	Benzidine	High
	4-aminobiphenyl	High

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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.	ethylene oxide propylene oxide	High High
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.	Naphthoquinone anthraquinone	Low High
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.	nitroglycerin dinitrotoluene	High High

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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.	Chloroform	High
	Fluoroacetate	High
	bromobenzene	High

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

Analytical Technique	LOD for the method	LOQ for method	Genotoxic impurity	Method Reference
HPLC-UV	0.1ppm	0.3 ppm	NDMA in Valsartan	ANSM Method reference no 18A0399-02 [19].
LC-MS/MS	0.01ppm	0.033 – 3.33 ppm	NDMA in Ranitidine method	US Food and Drug Administration (2019). LC-MS/MS method for determining NDMA in ranitidine drug substance and drug product [20].
LC-HRMS	0.01ppm	0.03 to 0.1ppm	NDMA in ARB Drugs	US FDA. - LC-HRMS NDMA detection method in metformin drug material and drug product. (2020). [21].
LC-ESI-HRMS	0.005ppm	0.01 to 0.1ppm	NDMA in Metformin	US FDA. LC-ESI-HRMS method for the determination of

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				Nitrosoamines impurities in metformin drug substance and drug product." (2020). [22].
GC-MS/MS	0.005ppm	0.008ppm	NDMA in Valsartan	US FDA method for Impurity Assay by GC-MS/MS of Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA), (2019). [23].
GC-MS-HS	0.05ppm	0.3ppm	NDMA in Valsartan	US FDA. GC/MS Headspace Method for NDMA Detection in Valsartan Drug Substances and Drug Products. [24].

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.
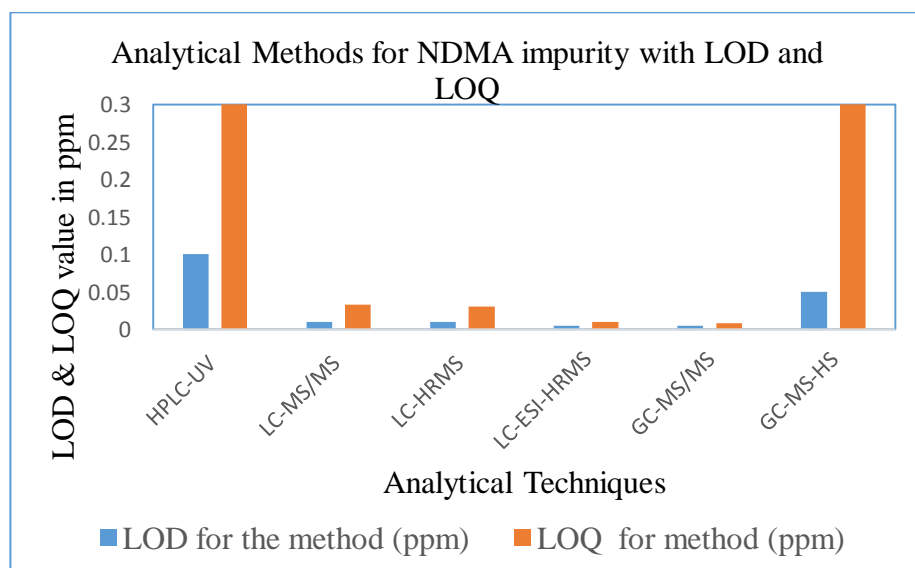

Figure 1: Analytical Methods for NDMA impurity with LOD and LOQ

The LOQ 0.03ppm sensitive methods are most suited for NDMA impurity analysis, according to FDA CGMP requirements.

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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.

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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

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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
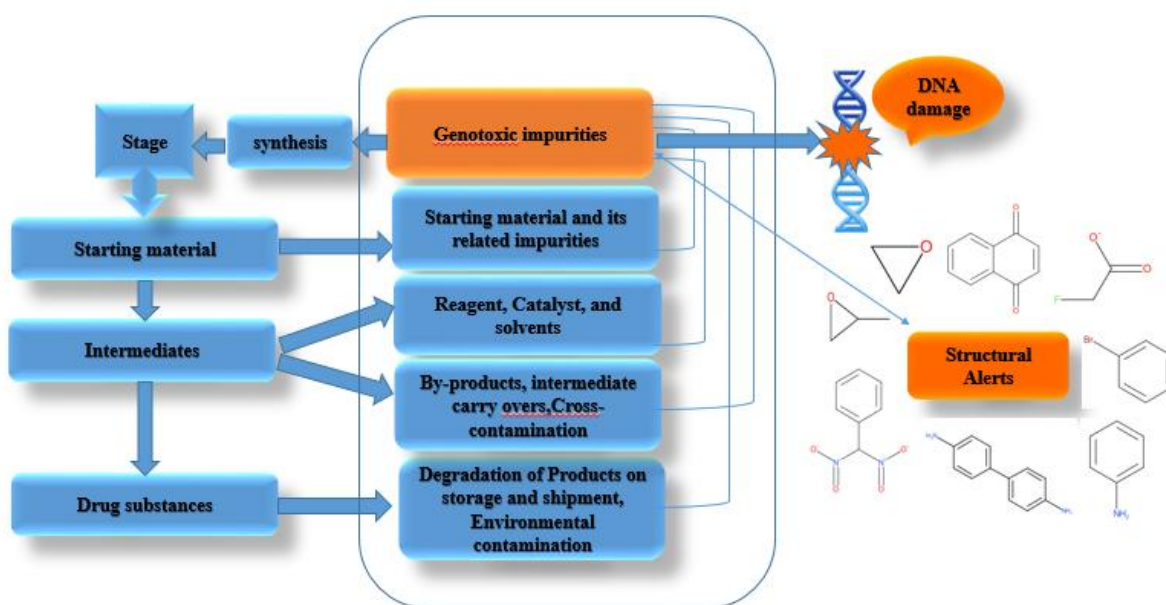
Figure 2: Sources of Genotoxic Impurities

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 (API's). 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

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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. Jamrógiewicz, 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)
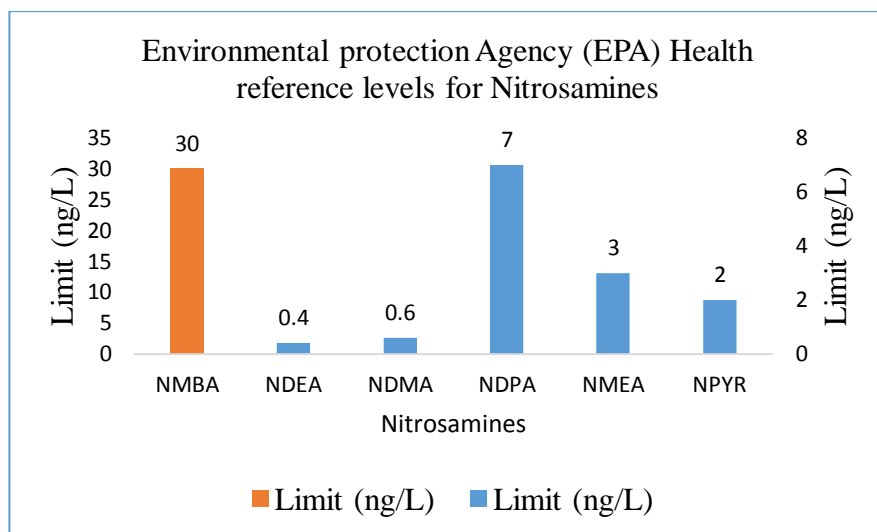
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Figure 3: Environmental Protection Agency (EPA) Health reference levels for Nitrosamines

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].

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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].

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

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N-Nitrosodi-n-propylamine	NDPA	0.186
Nitrosodibutylamine	NDBA	0.691
N-nitrosopyrrolidine	NPYR	0.799
N-Methyl-N´-nitro-N-nitrosoguanidin	MNNG	0.803
N-Methyl-N´-nitro-N-nitrosoguanidin	NMBA	0.982
N-Methyl-N´-nitro-N-nitrosoguanidin	NPIP	1.43
N-Nitrosodiethanolamine	NDELA	3.17
N,N-diisopropylethyl-N-ethylamine	DIPNA	0
N-nitrosodiphenylamine	NDPhA	167
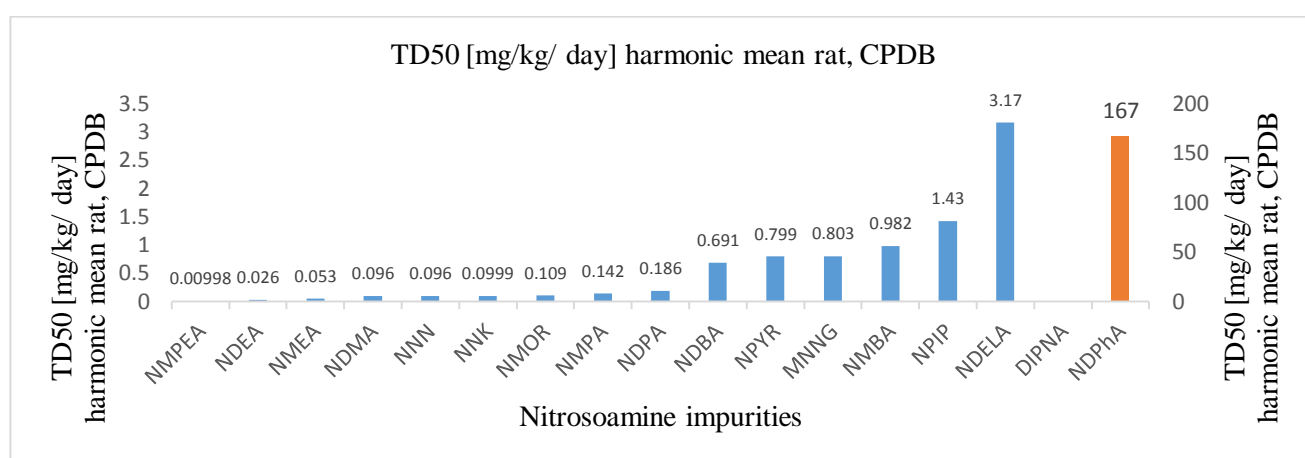
Figure 4: The TD50 [mg/kg/day] value was obtained from the harmonic mean of rats, data taken from Carcinogenic Potency Database (CPDB).

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

Chemical Name	TD 50 (mg/kg/day)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol	0.103
4-(Methylnitrosamino)-1-(3-pyridyl)-1-(butanone) [s]	0.0999
1-Nitroso-5,6-dihydrouracil	0.0983
N-Nitrosodimethylamine [s]	0.0959
N´-Nitrosonornicotine [s]	0.0957
Melphalan [s]	0.0938
N-Nitroso-*N*-methylurea [s]	0.0927
Nitrosoethylurethan	0.0904
Dinitrosohomopiperazine	0.0615

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Nitroso-1,2,3,6-tetrahydropyridine	0.0601
N-Nitroso-2,3-dihydroxypropyl-2-hydroxypropylamine [s]	0.0535
Triamcinolone acetonide	0.053
Nitrosoethylmethylamine	0.0503
Azoxymethane	0.0466
N-Nitrosomethyl-2-hydroxypropylamine	0.0463
Nitrosoheptamethyleneimine	0.0378
Chlorozotocin	0.0375
Nitroso-2,3-dihydroxypropyl-2-oxo-propylamine [s]	0.0352
Hexamethylphosphoramide	0.0344
N-Nitrosodiethylamine [s]	0.0265
Z-Ethyl-*O,N,N*-azoxyethane	0.022
Cadmium sulphate (1:1) [s]	0.0217
Z-Ethyl-*O,N,N*-azoxymethane	0.0189
N-Nitrosomethyl(2-oxopropyl) amine	0.0172
Aristolochic acid, sodium salt	0.0141
Cadmium chloride [s]	0.0136
Nitrogen mustard	0.0114
Nitroso-*N*-methyl-*N*-(2-phenyl) ethylamine	0.00998
Trenimon	0.00504
Bis-(chloromethyl)ether	0.00357
Aflatoxin B$_1$ [s]	0.0032
Aflatoxin, crude	0.00299
2-Azoxypropane	0.00268
Aflatoxicol	0.00247
Actinomycin D	0.00111
Mitomycin-C	0.00102
HCDD mixture	0.000596
1-Azoxypropane	0.000241
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	0.0000235

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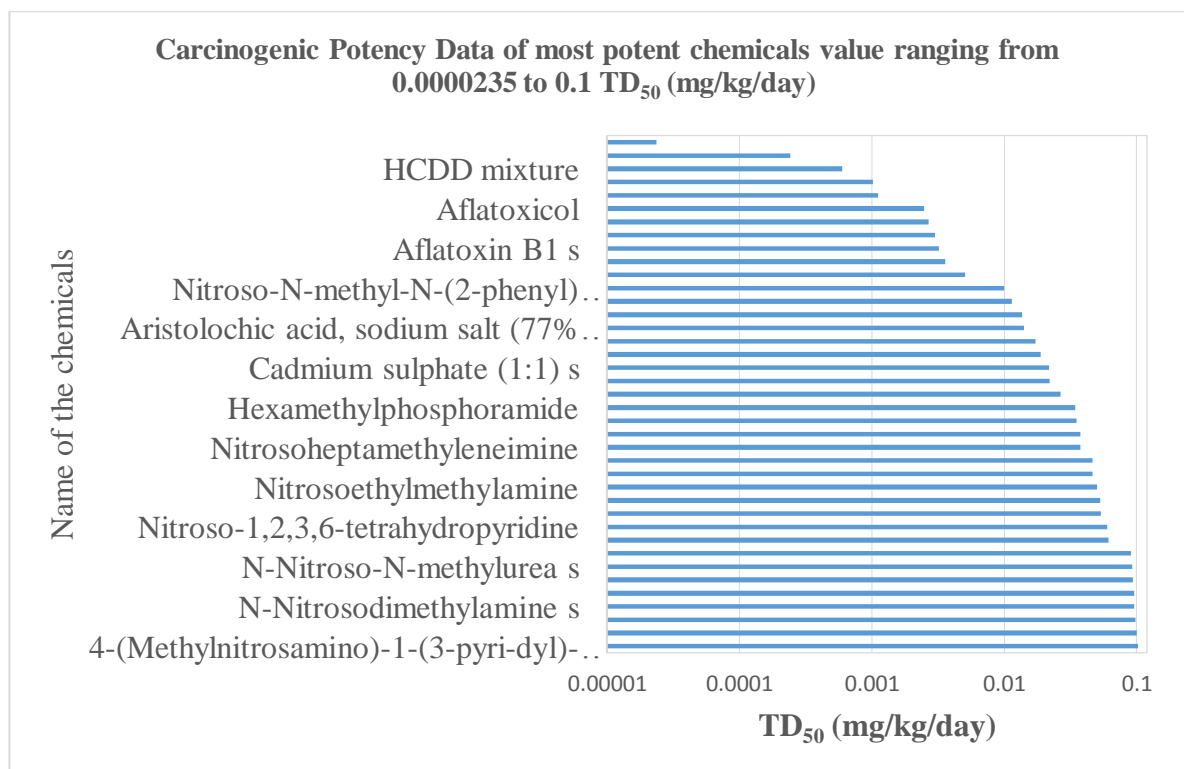*Section A-Research paper*

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).

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].

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

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Losartan	100	96	0.96	26.5	0.27	96	0.96***
Olmesartan	40	96	2.4	26.5	0.66	96	2.4
Telmisartan	80	96	1.2	26.5	0.33	96	1.2
Valsartan	320	96	0.3	26.5	0.083	96	0.3

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

Product	Method	Agency	GTI'S	Method LOQ Range
valsartan, losartan, irbesartan, olmesartan, candesartan	GC-MS/MS	Swissmedic OMCL	NDMA NDEA EIPNA DIPNA DPNA DBNA	LOQ 15 ppb
Ranitidine Drug Substance and Film Coated Tablets	LC-MS/MS	German OMCL at the Chemisches und Veterinär-Untersuchungsamt (CVUA)	NDMA	sample solution (0.5 ng/ml – 30 ng/ml) in drug substance and film-coated tablets (0,05 ppm – 3 ppm)

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Valsartan active substance	HPLC-UV	ANSM (French OMCL)	NDMA and NDEA	Valsartan NDMA LOQ 0.3ppm,
Sartan Drugs (valsartan, irbesartan, losartan, candesartan, and Olmesartan)	GC-MS-MS (Direct Injection)	Health Canada	NDMA and NDEA	NDMA LOQ 0.0054ppm NDEA LOQ 0.0073ppm
LOSARTAN Potassium	LC-MS/MS	edqm	NMBA	LOQ 28.6 ppb
Salbutamol Drug Substance	LC-MS/MS	Taiwan Food and Drug administration	N-Nitroso Salbutamol	(LOQ) for N-nitroso salbutamol is 0.025 μg/g
Sartan Drug Substances (losartan potassium drug substance)	HPLC	Taiwan Food and Drug Administration	Azido Compounds Test of (5-AMBBT) 5- (4'-((5-azidomethyl)-2-butyl- 4 -chloro-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-yl) - 1H-tetrazole	(LOQ) for 5-AMBBT is 8 μg/g
Medicines	GC-MS/MS	Taiwan Food and Drug Administration	12 nitrosamines such as N-nitroso dibutylamine (NDBA)	LOQ 0.05 μg/g N-Nitroso Diiso butylamine (NDiBA) LOQ 0.10 μg/g
Medicines	LC-MS/MS	Taiwan Food and Drug Administration	12 nitrosamines such as N-nitroso diethanolamine (NDELA)	LOQ 0.05 μg/g

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].

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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

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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 Orbi-trap 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].

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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].
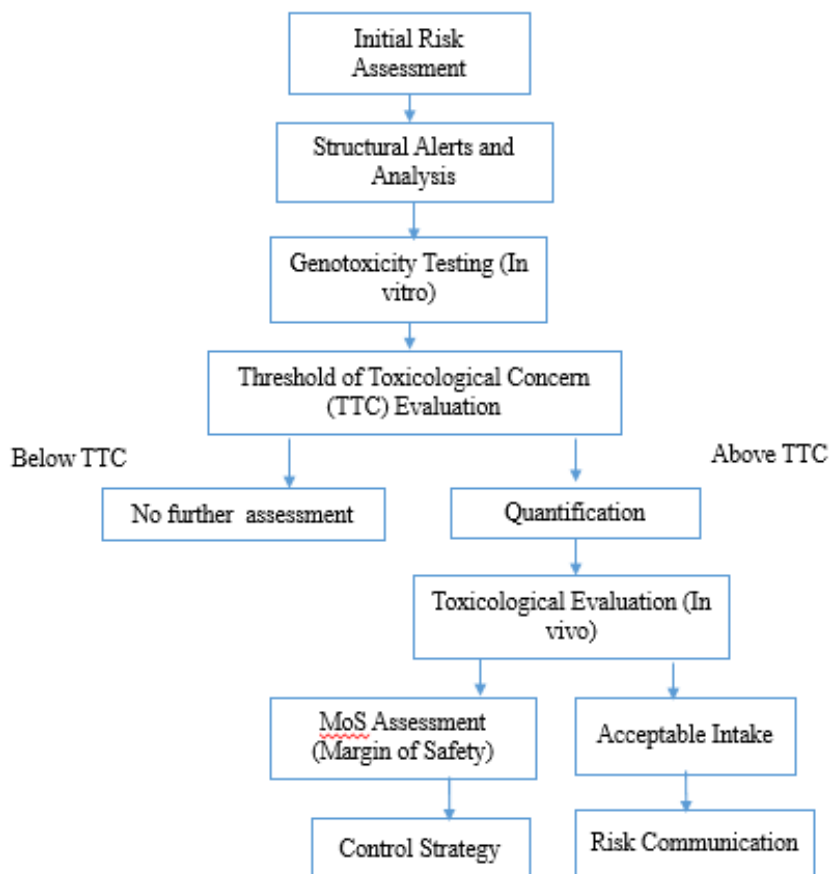
7.1 Risk Assessment and Identification:

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

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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 draught 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

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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.

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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

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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.

Acknowledgment

References

1) Müller L, Kikuchi Y, Probst G, Schechtman L, Shimada H, Sofuni T, Tweats D. ICH-harmonised guidances on genotoxicity testing of pharmaceuticals: evolution, reasoning and impact. Mutation Research/Reviews in Mutation Research. 1999 Jun 8;436(3):195-225.
https://doi.org/10.1016/S1383-5742(99)00004-6

2) Vogel EW, Natarajan AT. DNA damage and repair in somatic and germ cells in vivo. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1995 Aug 1;330(1-2):183-208.
https://doi.org/10.1016/0027-5107(95)00040-P

3) McGovern T, Jacobson-Kram D. Regulation of genotoxic and carcinogenic impurities in drug substances and products. TrAC Trends in Analytical Chemistry. 2006 Sep 1;25(8):790-5.
https://doi.org/10.1016/j.trac.2006.06.004

4) Jouyban A, Parsa H. Genotoxic impurities in pharmaceuticals. Toxicity and Drug testing. 2012 Feb 10:387-417.

5) US Food and Drug Administration. Current good manufacturing practice (CGMP) regulations. Zugriff am. 2018;28:2019.

6) Bharate SS. Critical analysis of drug product recalls due to nitrosamine impurities. Journal of Medicinal Chemistry. 2021 Mar 11;64(6):2923-36.
https://doi.org/10.1021/acs.jmedchem.0c02120

7) Vineis P, Pirastu R. Aromatic amines and cancer. Cancer Causes & Control. 1997 May;8:346-55.
https://doi.org/10.1023/A:1018453104303

12548

Eur. Chem. Bull. 2023,12(10), 12526-12564

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

8) Pira E, Piolatto G, Negri E, Romano C, Boffetta P, Lipworth L, McLaughlin JK, La Vecchia C. Bladder cancer mortality of workers exposed to aromatic amines: a 58-year follow-up. JNCI: Journal of the National Cancer Institute. 2010 Jul 21;102(14):1096-9.
https://doi.org/10.1093/jnci/djq214

9) Melnick RL. Carcinogenicity and mechanistic insights on the behavior of epoxides and epoxide- forming chemicals. Annals of the New York Academy of Sciences. 2002 Dec;982(1):177-89.
https://doi.org/10.1111/j.1749-6632.2002.tb04932.x

10) Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Morozzi G. Genotoxicity of alkene epoxides in human peripheral blood mononuclear cells and HL60 leukaemia cells evaluated with the comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2012 Aug 30;747(1):1-6.
https://doi.org/10.1016/j.mrgentox.2012.01.004

11) Xiong Y, Kaw HY, Zhu L, Wang W. Genotoxicity of quinone: an insight on DNA adducts and its LC-MS-based detection. Critical Reviews in Environmental Science and Technology. 2022 Dec 2;52(23):4217-40.
https://doi.org/10.1080/10643389.2021.2001276

12) Mueller SO, Schmitt M, Dekant W, Stopper H, Schlatter J, Schreier P, Lutz WK. Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. Food and chemical toxicology. 1999 May 1;37(5):481-91.
https://doi.org/10.1016/S0278-6915(99)00027-7

13) Rickert DE, Butterworth BE, Popp JA, Krahn DF. Dinitrotoluene: acute toxicity, oncogenicity, genotoxicity, and metabolism. CRC critical reviews in toxicology. 1984 Jan 1;13(3):217-34.
https://doi.org/10.3109/10408448409003373

14) Mirsalis JC, Hamm Jr TE, Sherrill JM, Butterworth BE. Role of gut flora in the genotoxicity of dinitrotoluene. Nature. 1982 Jan 28;295(5847):322-3.
https://doi.org/10.1038/295322a0

15) Bojar RM, Rastegar H, Payne DD, Harkness SH, England MR, Stetz JJ, Weiner B, Cleveland RJ. Methemoglobinemia from intravenous nitroglycerin: a word of caution. The Annals of thoracic surgery. 1987 Mar 1;43(3):332-4.
https://doi.org/10.1016/S0003-4975(10)60627-3

16) Mansouri A, Lurie AA. Methemoglobinemia. American journal of hematology. 1993 Jan;42(1):7-12.
https://doi.org/10.1002/ajh.2830420104

17) Fu PP, Von Tungeln LS, Chiu LH, Own ZY. Halogenated- polycyclic aromatic hydrocarbons: A class of Genotoxic environmental pollutants. Journal of Environmental Science & Health Part C. 1999 Nov 1;17(2):71-109.
https://doi.org/10.1080/10590509909373510

18) Fda US. Guidance for industry–control of nitrosamine impurities in human drugs. US Department of Health and Human Services, Food and Drug Administration. 2021.
https://www.fda.gov/media/141720/download

19) ANSM (French National Agency for Medicines and Health Products Safety) Determination of NDMA in Valsartan Active Substances and Finished Products by HPLC/UV Method Reference : 18A0399-02. 21 September 2018
https://www.edqm.eu/documents/52006/71923/Ad-hoc-projects-OMCL-Network-ansm-powdered-tablets-valsartan.pdf/a9bf0d81-f8da-9531-6de0-0268ab1614ad?t=1628667816207

20) US Food and Drug Administration. "Liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the determination of NDMA in ranitidine drug substance and drug product." (2019).

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

https://www.fda.gov/media/130801/download

21) US Food and Drug Administration. "Liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the determination of NDMA in metformin drug substance and drug product." (2020).
https://www.fda.gov/media/134914/download

22) US Food and Drug Administration. "Liquid chromatography-high resolution mass spectrometry (LC-ESI-HRMS) method for the determination of Nitrosoamines impurities in metformin drug substance and drug product." (2020).
https://www.fda.gov/media/138617/download

23) US Food and Drug Administration. "Combined Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA) Impurity Assay by GC-MS/MS." (2019).
https://www.fda.gov/media/123409/download

24) US Food and Drug Administration. "GC/MS Headspace Method for Detection of NDMA in Valsartan Drug Substances and Drug Products." (2019).
https://www.fda.gov/media/115965/download

25) Chittireddy HN, Kumar JS, Bhimireddy A, Shaik MR, Khan M, Khan M, Oh TH, Shaik B. Development and Validation of Analytical Method Using Gas Chromatography with Triple Quadrupole Mass Spectrometry for the Detection of Alkyl Halides as Potential Genotoxic Impurities in Posaconazole. Separations. 2023 May 6;10(5):295.
https://doi.org/10.3390/separations10050295

26) Park KM, Kim WM, Ahn SH, Lee HL, Hwang SH, Lee W, Hong J. Analytical methods to manage potential impurities in drug substances. Analytical Science and Technology. 2022;35(3):93-115.
https://doi.org/10.5806/AST.2022.35.3.93

27) Jahani M, Fazly Bazzaz BS, Akaberi M, Rajabi O, Hadizadeh F. Recent Progresses in analytical perspectives of degradation studies and impurity profiling in pharmaceutical developments: An updated review. Critical Reviews in Analytical Chemistry. 2021 Nov 18:1-22.
https://doi.org/10.1080/10408347.2021.2008226

28) Zhu Q, Scriba GK. Analysis of small molecule drugs, excipients and counter ions in pharmaceuticals by capillary electromigration methods–recent developments. Journal of Pharmaceutical and Biomedical Analysis. 2018 Jan 5;147:425-38.
https://doi.org/10.1016/j.jpba.2017.06.063

29) Ramachandra B. Development of impurity profiling methods using modern analytical techniques. Critical reviews in analytical chemistry. 2017 Jan 2;47(1):24-36.
https://doi.org/10.1080/10408347.2016.1169913

30) Jain M, Srivastava V, Kumar R, Dangi V, Hiriyanna SG, Kumar A, Kumar P. Determination of five potential genotoxic impurities in dalfampridine using liquid chromatography. Journal of pharmaceutical and biomedical analysis. 2017 Jan 30;133:27-31.
https://doi.org/10.1016/j.jpba.2016.10.013

31) Matveeva OA, Kovaleva EL. Modern approaches to estimating the content of genotoxic impurities in drugs (a review). Pharmaceutical Chemistry Journal. 2016 Feb;49:765-70.
https://doi.org/10.1007/s11094-016-1367-4

32) Al Azzam KM, Aboul-Enein HY. Recent advances in analysis of hazardous genotoxic impurities in pharmaceuticals by HPLC, GC, and CE. Journal of Liquid Chromatography & Related Technologies. 2016 Jan 2;39(1):1-7.
https://doi.org/10.1080/10826076.2015.1111794

33) Douša M, Klvaňˇa R, Doubský J, Srbek J, Richter J, Exner M, Gibala P. HILIC–MS determination of genotoxic impurity of 2-chloro-N-(2-chloroethyl) ethanamine in the vortioxetine manufacturing process. Journal of chromatographic science. 2016 Feb 1;54(2):119-24.
https://doi.org/10.1093/chromsci/bmv107

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

34) Wigman L, Zhang K, Kumar A. Analytical technologies for genotoxic impurities in pharmaceutical compounds. LCGC North America. 2015 May 1;33(5):344-59. https://www.chromatographyonline.com/view/analytical-technologies-genotoxic-impurities-pharmaceutical-compounds

35) Ho TD, Yehl PM, Chetwyn NP, Wang J, Anderson JL, Zhong Q. Determination of trace level genotoxic impurities in small molecule drug substances using conventional headspace gas chromatography with contemporary ionic liquid diluents and electron capture detection. Journal of Chromatography A. 2014 Sep 26;1361:217-28. https://doi.org/10.1016/j.chroma.2014.07.099

36) Lee H, editor. Pharmaceutical industry practices on genotoxic impurities. CRC Press; 2014 Aug 29.

37) Liu DQ, Kord AS. Analytical challenges in stability testing for genotoxic impurities. TrAC Trends in Analytical Chemistry. 2013 Sep 1;49:108-17. https://doi.org/10.1016/j.trac.2013.06.004

38) Batke M, Afrapoli FM, Kellner R, Rathman JF, Yang C, Cronin MT, Escher SE. Threshold of toxicological concern—an update for non-genotoxic carcinogens. Frontiers in Toxicology. 2021 Jun 24;3:688321. https://doi.org/10.3389/ftox.2021.688321

39) Boobis A, Brown P, Cronin MT, Edwards J, Galli CL, Goodman J, Jacobs A, Kirkland D, Luijten M, Marsaux C, Martin M. Origin of the TTC values for compounds that are genotoxic and/or carcinogenic and an approach for their re-evaluation. Critical Reviews in Toxicology. 2017 Sep 14;47(8):710-32. https://doi.org/10.1080/10408444.2017.1318822

40) Nohmi T. Thresholds of genotoxic and non-genotoxic carcinogens. Toxicological research. 2018 Oct;34:281-90. https://doi.org/10.5487/TR.2018.34.4.281

41) Cheeseman MA, Machuga EJ, Bailey AB. A tiered approach to threshold of regulation. Food and Chemical Toxicology. 1999 Apr 1;37(4):387-412. https://doi.org/10.1016/S0278-6915(99)00024-1

42) Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, Van Schothorst F, Vos JG, Würtzen G. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food and chemical toxicology. 2004 Jan 1;42(1):65-83. https://doi.org/10.1016/j.fct.2003.08.006

43) Munro IC, Renwick AG, Danielewska-Nikiel B. The threshold of toxicological concern (TTC) in risk assessment. Toxicology letters. 2008 Aug 15;180(2):151-6. https://doi.org/10.1016/j.toxlet.2008.05.006

44) Kroes R, Renwick AG, Feron V, Galli CL, Gibney M, Greim H, Guy RH, Lhuguenot JC, Van de Sandt JJ. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food and Chemical Toxicology. 2007 Dec 1;45(12):2533-62. https://doi.org/10.1016/j.fct.2007.06.021

45) Gold LS, Zeiger E. Handbook of carcinogenic potency and genotoxicity databases. CRC Press; 1996 Nov 26.

46) Regulska K, Matera-Witkiewicz A, Mikołajczyk A, Stanisz BJ. The Degradation Product of Ramipril Is Potentially Carcinogenic, Genotoxic and Mutagenic. Applied Sciences. 2023 Feb 12;13(4):2358. https://doi.org/10.3390/app13042358

47) Benigni R, Bassan A, Pavan M. In silico models for genotoxicity and drug regulation. Expert Opinion on Drug Metabolism & Toxicology. 2020 Aug 2;16(8):651-62. https://doi.org/10.1080/17425255.2020.1785428

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

48) Greene N, Dobo KL, Kenyon MO, Cheung J, Munzner J, Sobol Z, Sluggett G, Zelesky T, Sutter A, Wichard J. A practical application of two in silico systems for identification of potentially mutagenic impurities. Regulatory Toxicology and Pharmacology. 2015 Jul 1;72(2):335-49. https://doi.org/10.1016/j.yrtph.2015.05.008

49) Wichard JD. In silico prediction of genotoxicity. Food and Chemical Toxicology. 2017 Aug 1;106:595-9. https://doi.org/10.1016/j.fct.2016.12.013

50) Dobo KL, Greene N, Cyr MO, Caron S, Ku WW. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. Regulatory Toxicology and Pharmacology. 2006 Apr 1;44(3):282-93. https://doi.org/10.1016/j.yrtph.2006.01.004

51) Snodin DJ. Genotoxic impurities: from structural alerts to qualification. Organic process research & development. 2010 Jul 16;14(4):960-76. https://doi.org/10.1021/op100118e

52) Plošnik A, Vračko M, Sollner Dolenc M. Mutagenic and carcinogenic structural alerts and their mechanisms of action. Arhiv za higijenu rada i toksikologiju. 2016 Sep 22;67(3):169-82. https://doi.org/10.1515/aiht-2016-67-2801

53) Raillard SP, Bercu J, Baertschi SW, Riley CM. Prediction of drug degradation pathways leading to structural alerts for potential genotoxic impurities. Organic Process Research & Development. 2010 Jul 16;14(4):1015-20. https://doi.org/10.1021/op100007q

54) Clark, Kevin D., Cheng Zhang, and Jared L. Anderson. "Sample preparation for bioanalytical and pharmaceutical analysis." (2016): 11262-70. https://doi.org/10.1021/acs.analchem.6b02935

55) An J, Sun M, Bai L, Chen T, Liu DQ, Kord A. A practical derivatization LC/MS approach for determination of trace level alkyl sulfonates and dialkyl sulfates genotoxic impurities in drug substances. Journal of pharmaceutical and biomedical analysis. 2008 Nov 4;48(3):1006-10. https://doi.org/10.1016/j.jpba.2008.06.019

56) Bai L, Sun M, An J, Liu DQ, Chen TK, Kord AS. Enhancing the detection sensitivity of trace analysis of pharmaceutical genotoxic impurities by chemical derivatization and coordination ion spray-mass spectrometry. Journal of Chromatography A. 2010 Jan 15;1217(3):302-6. https://doi.org/10.1016/j.chroma.2009.11.048

57) Wang J, Yang S, Zhang K. A simple and sensitive method to analyze genotoxic impurity hydrazine in pharmaceutical materials. Journal of pharmaceutical and biomedical analysis. 2016 Jul 15;126:141-7. https://doi.org/10.1016/j.jpba.2016.04.038

58) Elder DP, Snodin D, Teasdale A. Control and analysis of hydrazine, hydrazides and hydrazones—genotoxic impurities in active pharmaceutical ingredients (APIs) and drug products. Journal of pharmaceutical and biomedical analysis. 2011 Apr 5;54(5):900-10. https://doi.org/10.1016/j.jpba.2010.11.007

59) Cappiello A, Famiglini G, Palma P, Termopoli V, Trufelli H. A new liquid chromatography–mass spectrometry approach for generic screening and quantitation of potential genotoxic alkylation compounds without derivatization. Journal of Chromatography A. 2012 Sep 14;1255:286-90. https://doi.org/10.1016/j.chroma.2011.12.068

60) Kumar T, Ramya M, Srinivasan V, Xavier N. A Simple and Direct LC–MS Method for Determination of Genotoxic Impurity Hydroxylamine in Pharmaceutical compounds. Journal of chromatographic science. 2017 Aug 1;55(7):683-9. https://doi.org/10.1093/chromsci/bmx019

61) Ho TD, Joshi MD, Silver MA, Anderson JL. Selective extraction of genotoxic impurities and structurally alerting compounds using polymeric ionic liquid sorbent coatings in solid-phase

12552

Eur. Chem. Bull. 2023,12(10), 12526-12564

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

microextraction: Alkyl halides and aromatics. Journal of Chromatography A. 2012 Jun 1;1240:29-44.
https://doi.org/10.1016/j.chroma.2012.03.080

62) Yu H, Ho TD, Anderson JL. Ionic liquid and polymeric ionic liquid coatings in solid-phase microextraction. TrAC Trends in Analytical Chemistry. 2013 Apr 1;45:219-32.
https://doi.org/10.1016/j.trac.2012.10.016

63) Cui Y, Liu D, Bian J, Yang Y, Zhao M, Jiang Y. Dispersive liquid-liquid microextraction with high-performance liquid chromatography for the analysis of 1, 4-benzodioxane-6-aldehyde in eliglustat tartrate active pharmaceutical ingredient. Journal of Pharmaceutical and Biomedical Analysis. 2020 Feb 5;179:112988.
https://doi.org/10.1016/j.jpba.2019.112988

64) Vogt FG, Kord AS. Development of quality-by-design analytical methods. Journal of pharmaceutical sciences. 2011 Mar 1;100(3):797-812.
https://doi.org/10.1002/jps.22325

65) Sun M, Liu DQ, Kord AS. A systematic method development strategy for determination of pharmaceutical genotoxic impurities. Organic Process Research & Development. 2010 Jul 16;14(4):977-85.
https://doi.org/10.1021/op100089p

66) Székely G, Henriques B, Gil M, Ramos A, Alvarez C. Design of experiments as a tool for LC–MS/MS method development for the trace analysis of the potentially genotoxic 4-dimethylaminopyridine impurity in glucocorticoids. Journal of pharmaceutical and biomedical analysis. 2012 Nov 1;70:251-8.
https://doi.org/10.1016/j.jpba.2012.07.006

67) Székely G, Henriques B, Gil M, Alvarez C. Experimental design for the optimization and robustness testing of a liquid chromatography tandem mass spectrometry method for the trace analysis of the potentially genotoxic 1, 3- diisopropylurea. Drug Testing and Analysis. 2014 Sep;6(9):898-908.
https://doi.org/10.1002/dta.1583

68) Grigori K, Loukas YL, Malenović A, Samara V, Kalaskani A, Dimovasili E, Kalovidouri M, Dotsikas Y. Chemometrically assisted development and validation of LC–MS/MS method for the analysis of potential genotoxic impurities in meropenem active pharmaceutical ingredient. Journal of Pharmaceutical and Biomedical Analysis. 2017 Oct 25;145:307-14.
https://doi.org/10.1016/j.jpba.2017.06.061

69) Jacobson-Kram D, McGovern T. Toxicological overview of impurities in pharmaceutical products. Advanced drug delivery reviews. 2007 Jan 10;59(1):38-42.
https://doi.org/10.1016/j.addr.2006.10.007

70) Szekely G, Amores de Sousa MC, Gil M, Castelo Ferreira F, Heggie W. Genotoxic impurities in pharmaceutical manufacturing: sources, regulations, and mitigation. Chemical reviews. 2015 Aug 26;115(16):8182-229.
https://doi.org/10.1021/cr300095f

71) Lee K, Yoo W, Jeong JH. Analytical Method Development for 19 Alkyl Halides as Potential Genotoxic Impurities by Analytical Quality by Design. Molecules. 2022 Jul 11;27(14):4437.
https://doi.org/10.3390/molecules27144437

72) Jamrógiewicz M, Wielgomas B. Detection of some volatile degradation products released during photoexposition of ranitidine in a solid state. Journal of pharmaceutical and biomedical analysis. 2013 Mar 25;76:177-82.
https://doi.org/10.1016/j.jpba.2012.12.019

73) Guideline IH. Impurities: Guideline for residual solvents Q3C (R5). Current Step. 2005 Nov;4:1-25.

12553

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

74) Stiborová M, Mikšanová M, Šulc M, Rýdlová H, Schmeiser HH, Frei E. Identification of a genotoxic mechanism for the carcinogenicity of the environmental pollutant and suspected human carcinogen o- anisidine. International journal of cancer. 2005 Sep 20;116(5):667-78. https://doi.org/10.1002/ijc.21122

75) Mani S, Bharagava RN. Exposure to crystal violet, its toxic, genotoxic and carcinogenic effects on environment and its degradation and detoxification for environmental safety. Reviews of Environmental Contamination and Toxicology Volume 237. 2016:71-104. https://doi.org/10.1007/978-3-319-23573-8_4

76) Hayden PJ, Armento A, Jackson GR, Kaluzhny Y, Klausner M. Utilization Of The Comet Assay For Assessment Of Chemical Genotoxicity Potential In The EpiAirway™ In Vitro Human Airway Model. InB52. TOXICOLOGY OF OCCUPATIONAL AND ENVIRONMENTAL DISEASE 2011 May (pp. A3267-A3267). American Thoracic Society. https://doi.org/10.1164/ajrccm-conference.2011.183.1_MeetingAbstracts.A3267

77) Ullah M. Selected Genotoxic Impurities Profiling During WFI Qualification to Control Carcinogenesis in Large Volume Parenterals. American Journal of Pharmacology and Toxicology. 2015 Jan 1;10(1):13. DOI: 10.3844/ajptsp.2015

78) Benford D, Bolger PM, Carthew P, Coulet M, DiNovi M, Leblanc JC, Renwick AG, Setzer W, Schlatter J, Smith B, Slob W. Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. Food and chemical Toxicology. 2010 Jan 1;48:S2-4. https://doi.org/10.1016/j.fct.2009.11.003

79) Masood F, Anjum R, Ahmad M, Malik A. Methods for genotoxicity testing of environmental pollutants. Environmental Protection Strategies for Sustainable Development. 2012:229-60. https://doi.org/10.1007/978-94-007-1591-2_7

80) Chmielińska K, Hubé D, Bausinger T, Simon M, Rivière G, Fauser P, Sanderson H. Environmental contamination with persistent cyclic mustard gas impurities and transformation products. Global Security: Health, Science and Policy. 2019 Jan 1;4(1):14-23. https://doi.org/10.1080/23779497.2019.1699848

81) Farmer PB, Singh R, Kaur B, Sram RJ, Binkova B, Kalina I, Popov TA, Garte S, Taioli E, Gabelova A, Cebulska-Wasilewska A. Molecular epidemiology studies of carcinogenic environmental pollutants: effects of polycyclic aromatic hydrocarbons (PAHs) in environmental pollution on exogenous and oxidative DNA damage. Mutation Research/Reviews in Mutation Research. 2003 Nov 1;544(2-3):397-402. https://doi.org/10.1016/j.mrrev.2003.09.002

82) Fu PP, Von Tungeln LS, Chiu LH, Own ZY. Halogenated- polycyclic aromatic hydrocarbons: A class of Genotoxic environmental pollutants. Journal of Environmental Science & Health Part C. 1999 Nov 1;17(2):71-109. https://doi.org/10.1080/10590509909373510

83) Vasseur P, Cossu-Leguille C. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere. 2006 Feb 1;62(7):1033-42. https://doi.org/10.1016/j.chemosphere.2005.05.043

84) Han MA, Kim JH, Song HS. Persistent organic pollutants, pesticides, and the risk of thyroid cancer: systematic review and meta-analysis. European Journal of Cancer Prevention. 2019 Jul 1;28(4):344-9. DOI: 10.1097/CEJ.0000000000000481

85) Elder D, Harvey J. Genotoxic Impurities: A Risk in Perspective. Genotoxic Impurities: Strategies for Identification and Control. 2011 Feb 14:193-218. DOI:10.1002/9780470929377

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

86) Guideline IH. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use S2 (R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Rockville, Maryland, US 2011.

87) Snodin DJ, McCrossen SD. Guidelines and pharmacopoeial standards for pharmaceutical impurities: overview and critical assessment. Regulatory Toxicology and Pharmacology. 2012 Jul 1;63(2):298-312.
https://doi.org/10.1016/j.yrtph.2012.03.016

88) Guideline IH. Assessment and control of dna reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva 2014 Jun 5.

89) Barber C, Amberg A, Custer L, Dobo KL, Glowienke S, Van Gompel J, Gutsell S, Harvey J, Honma M, Kenyon MO, Kruhlak N. Establishing best practise in the application of expert review of mutagenicity under ICH M7. Regulatory Toxicology and Pharmacology. 2015 Oct 1;73(1):367-77.
https://doi.org/10.1016/j.yrtph.2015.07.018

90) Amberg A, Andaya RV, Anger LT, Barber C, Beilke L, Bercu J, Bower D, Brigo A, Cammerer Z, Cross KP, Custer L. Principles and procedures for handling out-of-domain and indeterminate results as part of ICH M7 recommended (Q) SAR analyses. Regulatory toxicology and pharmacology. 2019 Mar 1;102:53-64.
https://doi.org/10.1016/j.yrtph.2018.12.007

91) Foster RS, Fowkes A, Cayley A, Thresher A, Werner AL, Barber CG, Kocks G, Tennant RE, Williams RV, Kane S, Stalford SA. The importance of expert review to clarify ambiguous situations for (Q) SAR predictions under ICH M7. Genes and Environment. 2020 Sep 22;42(1):27.
https://doi.org/10.1186/s41021-020-00166-y

92) Barber C, Hanser T, Judson P, Williams R. Distinguishing between expert and statistical systems for application under ICH M7. Regulatory Toxicology and Pharmacology. 2017 Mar 1;84:124-30.
https://doi.org/10.1016/j.yrtph.2016.12.012

93) Hasselgren C, Bercu J, Cayley A, Cross K, Glowienke S, Kruhlak N, Muster W, Nicolette J, Reddy MV, Saiakhov R, Dobo K. Management of pharmaceutical ICH M7 (Q) SAR predictions–the impact of model updates. Regulatory Toxicology and Pharmacology. 2020 Dec 1;118:104807.
https://doi.org/10.1016/j.yrtph.2020.104807

94) Teasdale A. ICH M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. ICH Quality Guidelines: An Implementation Guide. 2017 Sep 27:667-99.
https://doi.org/10.1002/9781118971147.ch24

95) Snodin D. ICH Guideline M7 on mutagenic impurities in pharmaceuticals. Regul. Rapp. 2017;14:5-9.
https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/8493114

96) Guideline IH. Impurities in new drug substances Q3A (R2). In Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland 2006 Oct 25 (Vol. 25).

97) USP C. 1663> Assessment of Extractables Associated with Pharmaceutical Packaging. Delivery Systems, USP.:1835-48.

98) Broschard TH, Glowienke S, Bruen US, Nagao LM, Teasdale A, Stults CL, Li KL, Iciek LA, Erexson G, Martin EA, Ball DJ. Assessing safety of extractables from materials and leachables in pharmaceuticals and biologics–Current challenges and approaches. Regulatory Toxicology and Pharmacology. 2016 Nov 1;81:201-11.
https://doi.org/10.1016/j.yrtph.2016.08.011

99) Bicker, M. "Comparative Extractable Studies for Injectables and Medical Devices Aligned with USP< 1663> and ISO 10993 Guidelines, ONdrugDelivery, Issue 120 (May 2021), pp 86–95. Matthias Bicker, Michael Müller, Marc Mittermüller, Daniel Haines and Uwe Rothhaar discuss

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Section A-Research paper

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/

100) Committee for Medicinal Products for Human Use. European Medicines Agency, 2007. Guideline on the limits of genotoxic impurities. EMEA/CHMP/QWP/251344; 2006.

101) Committee for Medicinal Products for Human Use (CHMP). European Medicines Agency, 2020. Assessment report. EMA/47245/2021; 2020.
https://www.ema.europa.eu/en/medicines/human/referrals/angiotensin-ii-receptor-antagonists-sartans-containing-tetrazole-group

102) Wichitnithad W, Nantaphol S, Noppakhunsomboon K, Rojsitthisak P. An update on the current status and prospects of nitrosation pathways and possible root causes of nitrosamine formation in various pharmaceuticals. Saudi Pharmaceutical Journal. 2022 Dec 24.
https://doi.org/10.1016/j.jsps.2022.12.010

103) Committee for Medicinal Products for Human Use(CHMP). European Medicines Agency, 2020. Assessment report. EMEA/369136/2020, 2020.
https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf

104) FDA U. Guidance for industry: Genotoxic and carcinogenic impurities in drug substances and products: Recommended approaches. Draft, December. 2008 Dec.

105) Food and Drug Administration. FDA updates and press announcements on angiotensin II receptor blocker (ARB) recalls (Valsartan, Losartan, and Irbesartan). Food and Drug Administration. Available online at: https://www. fda. gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcementsangiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan. 2019.

106) Fda US. Guidance for industry–control of nitrosamine impurities in human drugs. US Department of Health and Human Services, Food and Drug Administration. 2021.

107) Horne S, Vera MD, Nagavelli LR, Sayeed VA, Heckman L, Johnson D, Berger D, Yip YY, Krahn CL, Sizukusa LO, Rocha NF. Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals. Journal of Pharmaceutical Sciences. 2023 May 1;112(5):1166-82.
https://doi.org/10.1016/j.xphs.2022.12.022Get rights and content

108) Wang J, Yang S, Zhang K. A simple and sensitive method to analyze genotoxic impurity hydrazine in pharmaceutical materials. Journal of pharmaceutical and biomedical analysis. 2016 Jul 15;126:141-7.
https://doi.org/10.1016/j.jpba.2016.04.038

109) Jain M, Srivastava V, Kumar R, Dangi V, Hiriyanna SG, Kumar A, Kumar P. Determination of five potential genotoxic impurities in dalfampridine using liquid chromatography. Journal of pharmaceutical and biomedical analysis. 2017 Jan 30;133:27-31.
https://doi.org/10.1016/j.jpba.2016.10.013

110) Dejaegher B, Vander Heyden Y. HILIC methods in pharmaceutical analysis. Journal of separation science. 2010 Mar;33(6- 7):698-715.
https://doi.org/10.1002/jssc.200900742

111) Douša M, Doubský J, Srbek J. Utilization of photochemically induced fluorescence detection for HPLC determination of genotoxic impurities in the vortioxetine manufacturing process. Journal of chromatographic science. 2016 Oct 17;54(9):1625-30.
https://doi.org/10.1093/chromsci/bmw116

112) Poster DL, Schantz MM, Sander LC, Wise SA. Analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples: a critical review of gas chromatographic (GC) methods. Analytical and bioanalytical chemistry. 2006 Oct;386:859-81.
https://doi.org/10.1007/s00216-006-0771-0

12556

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

113) Grifoll M, Solanas AM, Bayona JM. Characterization of genotoxic components in sediments by mass spectrometric techniques combined with Salmonella/microsome test. Archives of environmental contamination and toxicology. 1990 Mar;19:175-84. https://doi.org/10.1007/BF01056084

114) Raman NV, Prasad AV, Reddy KR. Sensitive derivatization methods for the determination of genotoxic impurities in drug substances using hyphenated techniques. Journal of pharmaceutical and Biomedical Analysis. 2014 Feb 15;89:276-81. https://doi.org/10.1016/j.jpba.2013.11.013

115) K Vyas V, Ghate M, D Ukawala R. Recent advances in characterization of impurities-Use of hyphenated LC-MS technique. Current Pharmaceutical Analysis. 2010 Nov 1;6(4):299-306. https://doi.org/10.2174/157341210793292392

116) Zhao Y, Li J, Xie H, Li H, Chen X. Covalent organic nanospheres as a fiber coating for solid-phase microextraction of genotoxic impurities followed by analysis using GC-MS. Journal of Pharmaceutical Analysis. 2022 Aug 1;12(4):583-9. https://doi.org/10.1016/j.jpha.2021.12.002

117) El Deeb S, Wätzig H, Abd El- Hady D, Sänger- van de Griend C, Scriba GK. Recent advances in capillary electrophoretic migration techniques for pharmaceutical analysis (2013–2015). Electrophoresis. 2016 Jul;37(12):1591-608. https://doi.org/10.1002/elps.201600058

118) Řemínek R, Foret F. Capillary electrophoretic methods for quality control analyses of pharmaceuticals: A review. Electrophoresis. 2021 Jan;42(1-2):19-37. https://doi.org/10.1002/elps.202000185

119) Zeiger E. The test that changed the world: The Ames test and the regulation of chemicals. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2019 May 1;841:43-8. https://doi.org/10.1016/j.mrgentox.2019.05.007

120) Stead AG, Hasselblad V, Creason JP, Claxton L. Modeling the Ames test. Mutation Research/Environmental Mutagenesis and Related Subjects. 1981 Feb 1;85(1):13-27. https://doi.org/10.1016/0165-1161(81)90282-X

121) Cariello NF, Piegorsch WW. The Ames test: the two-fold rule revisited. Mutation Research/Genetic Toxicology. 1996 Jul 10;369(1-2):23-31. https://doi.org/10.1016/S0165-1218(96)90044-0

122) Vargas VM, Motta VE, Henriques JA. Mutagenic activity detected by the Ames test in river water under the influence of petrochemical industries. Mutation Research/Genetic Toxicology. 1993 Sep 1;319(1):31-45. https://doi.org/10.1016/0165-1218(93)90028-C

123) Kenyon MO, Cheung JR, Dobo KL, Ku WW. An evaluation of the sensitivity of the Ames assay to discern low-level mutagenic impurities. Regulatory Toxicology and Pharmacology. 2007 Jun 1;48(1):75-86. https://doi.org/10.1016/j.yrtph.2007.01.006

124) McCann J, Horn L, Kaldor J. An evaluation of Salmonella (Ames) test data in the published literature: application of statistical procedures and analysis of mutagenic potency. Mutation Research/Reviews in Genetic Toxicology. 1984 Jul 1;134(1):1-47. https://doi.org/10.1016/0165-1110(84)90013-7

125) McMahon RE, Cline JC, Thompson CZ. Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. Cancer Research. 1979 Mar 1;39(3):682-93. https://aacrjournals.org/cancerres/article/39/3/682/483489/Assay-of-855-Test-Chemicals-in-Ten-Tester-Strains

126) Levy DD, Zeiger E, Escobar PA, Hakura A, Bas-Jan M, Kato M, Moore MM, Sugiyama KI. Recommended criteria for the evaluation of bacterial mutagenicity data (Ames test). Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2019 Dec 1;848:403074.

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

https://doi.org/10.1016/j.mrgentox.2019.07.004

127) Hillebrecht A, Muster W, Brigo A, Kansy M, Weiser T, Singer T. Comparative evaluation of in silico systems for ames test mutagenicity prediction: scope and limitations. Chemical research in toxicology. 2011 Jun 20;24(6):843-54.
https://doi.org/10.1021/tx2000398

128) Benigni R, Bossa C. Structure alerts for carcinogenicity, and the Salmonella assay system: a novel insight through the chemical relational databases technology. Mutation Research/Reviews in Mutation Research. 2008 Sep 1;659(3):248-61.
https://doi.org/10.1016/j.mrrev.2008.05.003

129) Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K, Tsuda S. The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and US NTP Carcinogenicity Database. Critical reviews in toxicology. 2000 Jan 1;30(6):629-799.
https://doi.org/10.1080/10408440008951123

130) Benigni R, Bossa C, Richard AM, Yang C. A novel approach: chemical relational databases, and the role of the ISSCAN database on assessing chemical carcinogenicity. Annali dell'Istituto superiore di sanità. 2008 Jan 1;44(1):48-56.
https://europepmc.org/article/med/18469376

131) Gold LS, Sawyer CB, Magaw R, Backman GM, de Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, Pike MC. 2012 The carcinogenic potency database.

132) Gold LS, Slone TH, Bernstein L. Summary of carcinogenic potency and positivity for 492 rodent carcinogens in the carcinogenic potency database. Environmental Health Perspectives. 1989 Feb;79:259-72.
https://doi.org/10.1289/ehp.8979259

133) Gold LS, Manley NB, Slone TH, Rohrbach L, Garfinkel GB. Supplement to the Carcinogenic Potency Database (CPDB): results of animal bioassays published in the general literature through 1997 and by the National Toxicology Program in 1997–1998. Toxicological Sciences. 2005 Jun 1;85(2):747-808.
https://doi.org/10.1093/toxsci/kfi161

134) Ashby J, Tennant RW. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the US NTP. Mutation Research/Reviews in Genetic Toxicology. 1991 May 1;257(3):229-306.
https://doi.org/10.1016/0165-1110(91)90003-E

135) Bagni G, Osella D, Sturchio E, Mascini M. Deoxyribonucleic acid (DNA) biosensors for environmental risk assessment and drug studies. Analytica chimica acta. 2006 Jul 28;573:81-9.
https://doi.org/10.1016/j.aca.2006.03.085

136) Epa US. User's guide for TEST (version 5.1)(toxicity estimation software tool): a program to estimate toxicity from molecular structure. Chemical Characterization and Exposure Division Cincinnati O, ed. 2020.

137) Cassano A, Manganaro A, Martin T, Young D, Piclin N, Pintore M, Bigoni D, Benfenati E. CAESAR models for developmental toxicity. InChemistry Central Journal 2010 Jul (Vol. 4, pp. 1-11). Springer International Publishing.
https://doi.org/10.1186/1752-153X-4-S1-S4

138) OECD. Guidance document on the validation of (Q) SAR models. Paris, France. Organisation for Economic Cooperation and Development. Environmental Health and Safety Publications. Series on Testing and Assessment. No. 69. 2007:154.

139) Madden JC, Enoch SJ, Paini A, Cronin MT. A review of in silico tools as alternatives to animal testing: principles, resources and applications. Alternatives to Laboratory Animals. 2020 Jul;48(4):146-72.
https://doi.org/10.1177/0261192920965977

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

140) Marchant CA, Briggs KA, Long A. In silico tools for sharing data and knowledge on toxicity and metabolism: Derek for windows, meteor, and vitic. Toxicology mechanisms and methods. 2008 Jan 1;18(2-3):177-87.
https://doi.org/10.1080/15376510701857320

141) Valerio Jr LG. In silico toxicology for the pharmaceutical sciences. Toxicology and applied pharmacology. 2009 Dec 15;241(3):356-70.
https://doi.org/10.1016/j.taap.2009.08.022

142) Madden JC, Webb S, Enoch SJ, Colley HE, Murdoch C, Shipley R, Sharma P, Yang C, Cronin MT. In silico prediction of skin metabolism and its implication in toxicity assessment. Computational Toxicology. 2017 Aug 1;3:44-57.
https://doi.org/10.1016/j.comtox.2017.07.001

143) Marzo M, Kulkarni S, Manganaro A, Roncaglioni A, Wu S, Barton-Maclaren TS, Lester C, Benfenati E. Integrating in silico models to enhance predictivity for developmental toxicity. Toxicology. 2016 Aug 31;370:127-37.
https://doi.org/10.1016/j.tox.2016.09.015

144) Contrera JF. Validation of Toxtree and SciQSAR in silico predictive software using a publicly available benchmark mutagenicity database and their applicability for the qualification of impurities in pharmaceuticals. Regulatory Toxicology and Pharmacology. 2013 Nov 1;67(2):285-93.
https://doi.org/10.1016/j.yrtph.2013.08.008

145) Worth A, Lapenna S, Lo Piparo E, Mostrag-Szlichtyng A, Serafimova R. The applicability of software tools for genotoxicity and carcinogenicity prediction: case studies relevant to the assessment of pesticides. JRC scientific and technical reports. EC Joint Research Centre Institute for Health and Consumer Protection, Ispra. 2010:18-9.

146) Lim CK, Yuan ZX, Jones RM, White IN, Smith LL. Identification and mechanism of formation of potentially genotoxic metabolites of tamoxifen: study by LC-MS/MS. Journal of pharmaceutical and biomedical analysis. 1997 Jun 1;15(9-10):1335-42.
https://doi.org/10.1016/S0731-7085(96)02007-9

147) Chidella KS, Dasari VB, Anireddy J. Ultra-sensitive LC-MS/MS method for the trace level quantification of six potential genotoxic nitrosamine impurities in telmisartan. American Journal of Analytical Chemistry. 2021 Jun 10;12(6):227-40.
DOI: 10.4236/ajac.2021.126014

148) Van Wijk AM, Niederländer HA, Siebum AH, Vervaart MA, De Jong GJ. A new derivatization reagent for LC–MS/MS screening of potential genotoxic alkylation compounds. Journal of pharmaceutical and biomedical analysis. 2013 Feb 23;74:133-40.
https://doi.org/10.1016/j.jpba.2012.10.004

149) Hutzler C, Luch A, Filser JG. Analysis of carcinogenic polycyclic aromatic hydrocarbons in complex environmental mixtures by LC-APPI-MS/MS. Analytica Chimica Acta. 2011 Sep 30;702(2):218-24.
https://doi.org/10.1016/j.aca.2011.07.003

150) Manchuri KM, Shaik MA, Gopireddy VS. A Novel UHPLC–MS/MS Method Development and Validation for Identification and Quantification of Genotoxic Impurity Bis (2-Chloroethyl) Amine in Aripiprazole Drug Substance. Chromatographia. 2022 Feb;85(2):137-46.
https://doi.org/10.1007/s10337-021-04123-x

151) Chen Y, Wu S, Yang Q. Development and validation of LC-MS/MS for analyzing potential genotoxic impurities in Pantoprazole starting materials. Journal of Analytical Methods in Chemistry. 2020 Mar 9;2020.
https://doi.org/10.1155/2020/6597363

152) Guo T, Shi Y, Zheng L, Feng F, Zheng F, Liu W. Rapid and simultaneous determination of sulfonate ester genotoxic impurities in drug substance by liquid chromatography coupled to

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

tandem mass spectrometry: Comparison of different ionization modes. Journal of chromatography A. 2014 Aug 15;1355:73-9.
https://doi.org/10.1016/j.chroma.2014.05.079

153) Yenugu VM, Ambavaram VB, Moniruzzaman M, Madhavi G. A simple, sensitive, and straightforward LC–MS approach for rapid analysis of three potential genotoxic impurities in rabeprazole formulations. Journal of separation science. 2018 Nov;41(21):3966-73.
https://doi.org/10.1002/jssc.201800626

154) Wang T, Yang H, Yang J, Guo N, Wu G, Xu X, An M. Quantitative Determination of Four Potential Genotoxic Impurities in the Active Pharmaceutical Ingredients in TSD-1 Using UPLC-MS/MS. Molecules. 2022 Jun 27;27(13):4129.
https://doi.org/10.3390/molecules27134129

155) Li S, Dong L, Tang K, Lan Z, Liu R, Wang Y, Wang R, Lin H. Simultaneous and trace level quantification of two potential genotoxic impurities in valsartan drug substance using UPLC-MS/MS. Journal of Pharmaceutical and Biomedical Analysis. 2022 Apr 1;212:114630.
https://doi.org/10.1016/j.jpba.2022.114630

156) Chittireddy HN, Kumar JS, Bhimireddy A, Shaik MR, Hatshan MR, Khan M, Alwarthan A, Shaik B. Development and Validation for Quantitative Determination of Genotoxic Impurity in Gemfibrozil by Gas Chromatography with Mass Spectrometry. Separations. 2023 Feb 21;10(3):145.
https://doi.org/10.3390/separations10030145

157) Lachenmeier DW, Frank W, Kuballa T. Application of tandem mass spectrometry combined with gas chromatography to the routine analysis of ethyl carbamate in stone- fruit spirits. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up- to- the- Minute Research in Mass Spectrometry. 2005 Jan 30;19(2):108-12.
https://doi.org/10.1002/rcm.1755

158) Lacroix C, Le Cuff N, Receveur J, Moraga D, Auffret M, Guyomarch J. Development of an innovative and "green" stir bar sorptive extraction–thermal desorption–gas chromatography–tandem mass spectrometry method for quantification of polycyclic aromatic hydrocarbons in marine biota. Journal of Chromatography A. 2014 Jul 4;1349:1-0.
https://doi.org/10.1016/j.chroma.2014.04.094

159) Atha DH, Coskun E, Erdem O, Tona A, Reipa V, Nelson BC. Genotoxic effects of etoposide, bleomycin, and ethyl methanesulfonate on cultured CHO cells: Analysis by GC-MS/MS and comet assay. Journal of nucleic acids. 2020 Jul 30;2020.
https://doi.org/10.1155/2020/8810105

160) Ankarberg-Lindgren C, Dahlgren J, Andersson MX. High-sensitivity quantification of serum androstenedione, testosterone, dihydrotestosterone, estrone and estradiol by gas chromatography–tandem mass spectrometry with sex-and puberty-specific reference intervals. The Journal of steroid biochemistry and molecular biology. 2018 Oct 1;183:116-24.
https://doi.org/10.1016/j.jsbmb.2018.06.005

161) Liu J, Xie B, Mai B, Cai Q, He R, Guo D, Zhang Z, Fan J, Zhang W. Development of a sensitive and stable GC-MS/MS method for simultaneous determination of four N-nitrosamine genotoxic impurities in sartan substances. Journal of analytical science and technology. 2021 Dec;12(1):1-8.
https://doi.org/10.1186/s40543-020-00254-2

162) Arrebola FJ, Frenich AG, González Rodríguez MJ, Bolaños PP, Martínez Vidal JL. Determination of polycyclic aromatic hydrocarbons in olive oil by a completely automated headspace technique coupled to gas chromatography- mass spectrometry. Journal of mass spectrometry. 2006 Jun;41(6):822-9.
https://doi.org/10.1002/jms.1040

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

163) Tummala SR, Amgoth KP. Development of GC-MS/MS Method for Simultaneous Estimation of Four Nitrosoamine Genotoxic Impurities in Valsartan. Turkish Journal of Pharmaceutical Sciences. 2022 Aug;19(4):455.
doi: 10.4274/tjps.galenos.2021.17702

164) Ahirrao VK, Jadhav RA, Rane VP, Bhamare HR, Yeole RD. Time-dependent selected reaction monitoring-based GC-MS/MS method for estimation of genotoxic impurities in new antibacterial agent: alalevonadifloxacin mesylate. Journal of Analytical Science and Technology. 2020 Dec;11:1-9.
https://doi.org/10.1186/s40543-020-00214-w

165) Chidella KS, Dasari VB, Anireddy J. Ultra-sensitive LC-MS/MS method for the trace level quantification of six potential genotoxic nitrosamine impurities in telmisartan. American Journal of Analytical Chemistry. 2021 Jun 10;12(6):227-40.
DOI: 10.4236/ajac.2021.126014

166) Bo LI, Tong ZH, Qingsheng ZH, Huihong FA. Determination of 7, N-nitrosoamines in Metformin Hydrochloride Sustained Release Tablets by LC-HRMS. Chinese Journal of Pharmacovigilance. 2021 May 15;18(5):454.

167) Bo LI, Xintong CH, Qingsheng ZH, Huihong FA. Determination of contents of NDMA in ranitidine hydrochloride substances and products by LC-HRMS. Chinese Journal of Pharmacovigilance. 2023 Feb 15;20(2):168.

168) Khorolskiy M, Ramenskaya G, Vlasov A, Perederyaev O, Maslennikova N. Development and validation of four nitrosamine impurities determination method in medicines of valsartan, losartan, and irbesartan with HPLC-MS/MS (APCI). Iranian Journal of Pharmaceutical Research: IJPR. 2021;20(3):541.
doi: 10.22037/ijpr.2021.115102.15195

169) Christensen A, Östman C, Westerholm R. Ultrasound-assisted extraction and on-line LC–GC–MS for determination of polycyclic aromatic hydrocarbons (PAH) in urban dust and diesel particulate matter. Analytical and Bioanalytical Chemistry. 2005 Mar;381:1206-16.
https://doi.org/10.1007/s00216-005-3065-z

170) Östman C, Nilsson U. Coupled LC- GC- MS for on- line clean- up, separation, and identification of chlorinated polycyclic aromatic hydrocarbons at picogram levels in urban air. Journal of High Resolution Chromatography. 1992 Nov;15(11):745-50.
https://doi.org/10.1002/jhrc.1240151109

171) Tsizin S, Fialkov AB, Amirav A. Electron ionization mass spectrometry for both liquid and gas chromatography in one system without the need for hardware adjustments. Journal of the American Society for Mass Spectrometry. 2020 Jun 16;31(8):1713-21.
https://doi.org/10.1021/jasms.0c00136

172) Carr JE, Dill AE, Kwok K, Carnahan JW, Webster GK. LC-ICP-MS for nonmetal selective detection of pharmaceuticals. Current Pharmaceutical Analysis. 2008 Nov 1;4(4):206-14.
DOI: https://doi.org/10.2174/157341208786306234

173) Pereira AS, Schelfaut M, Lynen F, Sandra P. Design and evaluation of a multi-detection system composed of ultraviolet, evaporative light scattering and inductively coupled plasma mass spectrometry detection for the analysis of pharmaceuticals by liquid chromatography. Journal of Chromatography A. 2008 Mar 21;1185(1):78-84.
https://doi.org/10.1016/j.chroma.2008.01.030

174) Harigaya K, Yamada H, Horimoto S, Nishi H, Haginaka J. Sensitive quantitation of residual phenylhydrazine in antipyrine by LC-ICP-MS with iodo derivatization. Analytical Sciences. 2014 Aug;30(8):845-50.
https://doi.org/10.2116/analsci.30.845

175) Klencsár B, Li S, Balcaen L, Vanhaecke F. High-performance liquid chromatography coupled to inductively coupled plasma–Mass spectrometry (HPLC-ICP-MS) for quantitative metabolite profiling of non-metal drugs. TrAC Trends in Analytical Chemistry. 2018 Jul 1;104:118-34.

12561

Eur. Chem. Bull. 2023,12(10), 12526-12564

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

https://doi.org/10.1016/j.trac.2017.09.020

176) Okina M, Yoshida K, Kuroda K, Wanibuchi H, Fukushima S, Endo G. Determination of trivalent methylated arsenicals in rat urine by liquid chromatography–inductively coupled plasma mass spectrometry after solvent extraction. Journal of Chromatography B. 2004 Jan 25;799(2):209-15. https://doi.org/10.1016/j.jchromb.2003.10.028

177) Wilczewska K, Kot-Wasik A, Namieśnik J. LC-MS and LC-NMR as Complementary Techniques for the Determination of Pharmaceuticals in Dosage Formulations. Critical Reviews in Analytical Chemistry. 2013 Jul 1;43(3):148-75.
https://doi.org/10.1080/10408347.2013.810459

178) Singh S, Handa T, Narayanam M, Sahu A, Junwal M, Shah RP. A critical review on the use of modern sophisticated hyphenated tools in the characterization of impurities and degradation products. Journal of pharmaceutical and biomedical analysis. 2012 Oct 1;69:148-73.
https://doi.org/10.1016/j.jpba.2012.03.044

179) Maggio RM, Calvo NL, Vignaduzzo SE, Kaufman TS. Pharmaceutical impurities and degradation products: Uses and applications of NMR techniques. Journal of pharmaceutical and biomedical analysis. 2014 Dec 1;101:102-22.
https://doi.org/10.1016/j.jpba.2014.04.016

180) Kamboj S, Kamboj N, K Rawal R, Thakkar A, R Bhardwaj T. A compendium of techniques for the analysis of pharmaceutical impurities. Current Pharmaceutical Analysis. 2014 May 1;10(2):145-60.
DOI: 10.2174/1573412910666140220003500

181) Gonnella NC. LC-NMR: Expanding the limits of structure elucidation. CRC Press; 2020 Jan 15.

182) Blaženović I, Kind T, Ji J, Fiehn O. Software tools and approaches for compound identification of LC-MS/MS data in metabolomics. Metabolites. 2018 May 10;8(2):31.
 https://doi.org/10.3390/metabo8020031

183) Damiani T, Bonciarelli S, Thallinger GG, Koehler N, Krettler CA, Salihoglu AK, Korf A, Pauling JK, Pluskal T, Ni Z, Goracci L. Software and computational tools for LC-MS-based epilipidomics: Challenges and solutions. Analytical chemistry. 2023 Jan 10;95(1):287-303.
https://doi.org/10.1021/acs.analchem.2c04406

184) Müller L, Mauthe RJ, Riley CM, Andino MM, De Antonis D, Beels C, DeGeorge J, De Knaep AG, Ellison D, Fagerland JA, Frank R. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regulatory Toxicology and Pharmacology. 2006 Apr 1;44(3):198-211.
https://doi.org/10.1016/j.yrtph.2005.12.001

185) Barber C, Antonucci V, Baumann JC, Brown R, Covey-Crump E, Elder D, Elliott E, Fennell JW, Gallou F, Ide ND, Jordine G. A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies. Regulatory Toxicology and Pharmacology. 2017 Nov 1;90:22-8.
https://doi.org/10.1016/j.yrtph.2017.08.008

186) Teasdale A, Elder D, Chang SJ, Wang S, Thompson R, Benz N, Sanchez Flores IH. Risk assessment of genotoxic impurities in new chemical entities: strategies to demonstrate control. Organic Process Research & Development. 2013 Feb 15;17(2):221-30.
https://doi.org/10.1021/op300268u

187) Teasdale A. ICH M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. ICH Quality Guidelines: An Implementation Guide. 2017 Sep 27:667-99.
https://doi.org/10.1002/9781118971147.ch24

188) Raman NV, Prasad AV, Reddy KR. Strategies for the identification, control and determination of genotoxic impurities in drug substances: A pharmaceutical industry perspective. Journal of pharmaceutical and biomedical analysis. 2011 Jun 25;55(4):662-7.
https://doi.org/10.1016/j.jpba.2010.11.039

12562

Eur. Chem. Bull. 2023,12(10), 12526-12564

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

189) Giordani A, Kobel W, Gally HU. Overall impact of the regulatory requirements for genotoxic impurities on the drug development process. European Journal of pharmaceutical sciences. 2011 May 18;43(1-2):1-5.
https://doi.org/10.1016/j.ejps.2011.03.004

190) Robinson DI. Control of genotoxic impurities in active pharmaceutical ingredients: a review and perspective. Organic Process Research & Development. 2010 Jul 16;14(4):946-59.
https://doi.org/10.1021/op900341a

191) Looker AR, Ryan MP, Neubert-Langille BJ, Naji R. Risk assessment of potentially genotoxic impurities within the framework of quality by design. Organic Process Research & Development. 2010 Jul 16;14(4):1032-6.
https://doi.org/10.1021/op900338g

192) Guideline IH. Assessment and control of dna reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7. InInternational conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva 2014 Jun 5.

193) MacGregor JT, Frötschl R, White PA, Crump KS, Eastmond DA, Fukushima S, Guérard M, Hayashi M, Soeteman-Hernández LG, Johnson GE, Kasamatsu T. IWGT report on quantitative approaches to genotoxicity risk assessment II. Use of point-of-departure (PoD) metrics in defining acceptable exposure limits and assessing human risk. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2015 May 1;783:66-78.
https://doi.org/10.1016/j.mrgentox.2014.10.008

194) Johnson GE, Soeteman- Hernández LG, Gollapudi BB, Bodger OG, Dearfield KL, Heflich RH, Hixon JG, Lovell DP, MacGregor JT, Pottenger LH, Thompson CM. Derivation of point of departure (PoD) estimates in genetic toxicology studies and their potential applications in risk assessment. Environmental and molecular mutagenesis. 2014 Oct;55(8):609-23.
https://doi.org/10.1002/em.21870

195) Snodin DJ. Mutagenic impurities in pharmaceuticals: A critical assessment of the cohort of concern with a focus on N-nitrosamines. Regulatory Toxicology and Pharmacology. 2023 Apr 26:105403.
https://doi.org/10.1016/j.yrtph.2023.105403

196) Humfrey CD. Recent developments in the risk assessment of potentially genotoxic impurities in pharmaceutical drug substances. Toxicological sciences. 2007 Nov 1;100(1):24-8.
https://doi.org/10.1093/toxsci/kfm173

197) Reddy AV, Jaafar J, Umar K, Majid ZA, Aris AB, Talib J, Madhavi G. Identification, control strategies, and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: A comprehensive review. Journal of Separation Science. 2015 Mar;38(5):764-79.
https://doi.org/10.1002/jssc.201401143

198) Pierson DA, Olsen BA, Robbins DK, DeVries KM, Varie DL. Approaches to assessment, testing decisions, and analytical determination of genotoxic impurities in drug substances. Organic Process Research & Development. 2009 Mar 20;13(2):285-91.
https://doi.org/10.1021/op8002129

199) Swenberg JA, Lu K, Moeller BC, Gao L, Upton PB, Nakamura J, Starr TB. Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology, and risk assessment. Toxicological sciences. 2011 Mar 1;120(suppl_1):S130-45.
https://doi.org/10.1093/toxsci/kfq371

200) Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacology & therapeutics. 1996 Jan 1;71(1-2):57-81.
https://doi.org/10.1016/0163-7258(96)00062-9

201) La DK, Swenberg JA. DNA adducts: biological markers of exposure and potential applications to risk assessment. Mutation Research/Reviews in Genetic Toxicology. 1996 Sep 1;365(1-3):129-46.

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Section A-Research paper

https://doi.org/10.1016/S0165-1110(96)90017-2

202) Jarabek AM, Pottenger LH, Andrews LS, Casciano D, Embry MR, Kim JH, Preston RJ, Reddy MV, Schoeny R, Shuker D, Skare J. Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. Critical reviews in toxicology. 2009 Sep 1;39(8):659-78. https://doi.org/10.1080/10408440903164155

203) Choy WN. A review of the dose-response induction of DNA adducts by aflatoxin B1 and its implications to quantitative cancer-risk assessment. Mutation Research/Reviews in Genetic Toxicology. 1993 Mar 1;296(3):181-98. https://doi.org/10.1016/0165-1110(93)90010-K

204) Goldstein LS, Weyand EH, Safe S, Steinberg M, Culp SJ, Gaylor DW, Beland FA, Rodriguez LV. Tumors and DNA adducts in mice exposed to benzo [a] pyrene and coal tars: implications for risk assessment. Environmental Health Perspectives. 1998 Dec;106(suppl 6):1325-30. https://doi.org/10.1289/ehp.98106s61325

205) Kowtharapu LP, Katari NK, Muchakayala SK, Pydimarry SP, Rekulapally VK, Sandoval CA. QbD green analytical procedure for Novel study of a genotoxic and carcinogenic compound trace determination in physiological solution compatibility. Sustainable Chemistry and Pharmacy. 2023 Jun 1;33:101079. https://doi.org/10.1016/j.scp.2023.101079

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Eur. Chem. Bull. 2023,12(10), 12526-12564