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Regulatory Considerations of Pharmaceutical Impurities with Emphasis on Genotoxic Impurities

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ABSTRACT

Over the past decade, significant changes have occurred in the approach to drug impurity profiling, as reflected in pharmacopeial and regulatory standards. This review provides an in-depth exploration of impurity profiling, focusing on the regulatory requirements. It offers detailed insights into various impurities such as residual solvents, water impurities, elemental contaminants, and carcinogenic substances, with special attention to genotoxic impurities. To meet the required quality standards, several pharmacopoeias have developed monographs. Regulatory organizations, including ICH, EMEA, USFDA, and the European Pharmacopeia, provide guidelines to minimize contaminants in pharmaceutical products. To detect and prevent impurities, the pharmaceutical and research sectors widely use analytical techniques such as HPLC, LC/MS, and GC/MS. This review also highlights the critical role of understanding genotoxic impurities as an essential aspect of a drug's impurity profile.

Keywords: Regulatory guidelines, Impurity, Mutagenic, Genotoxic, Carcinogenic

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Introduction

In the last decade, there has been increasing interest in drug impurity profiling within the pharmaceutical industry. Even trace amounts of impurities can significantly affect the safety and efficacy of drug products. To ensure that drug substances and products meet required quality standards, various pharmacopeias, including the British Pharmacopoeia, Indian Pharmacopoeia, European Pharmacopoeia, and United States Pharmacopoeia (USP), have developed specific monographs [1-3]. While some impurities may possess medicinal or toxic properties, their presence in drug materials can compromise the product's purity [4]. The International Council for Harmonisation (ICH) defines an impurity as any substance found in a drug product or its components that is not the active pharmaceutical ingredient (API), impacting the purity of the drug or its active compounds. To establish appropriate regulatory standards and management practices, it is essential to categorize the sources of impurities. As public and media concerns about medication safety grow, the importance of managing pharmaceutical contaminants has intensified. Regulatory guidance is available from both domestic and international bodies and is well documented in recent books and journal articles [5, 6].

This review article examines contaminants present in drug substances and pharmaceutical products, offering valuable insights into different impurity types, their classification, sources, and methods for their identification, isolation, and characterization. It also highlights the identification and categorization of genotoxic impurities.

Results and Discussion

Impurities in the pharmaceutical industry

The importance of impurity profiling in APIs has gained recognition due to the potential impact of impurities on the quality and safety of pharmaceutical products. Identifying, isolating, and quantifying these impurities is a crucial part of drug development and regulatory assessments. Pharmaceutical contaminants may be present alongside APIs or arise during manufacturing or product aging. Even at low concentrations, these contaminants can influence a drug's effectiveness and safety. While research into impurities has expanded, challenges remain in developing effective techniques to identify degradation products and process-related contaminants. This study aims to provide an overview of key international regulatory standards related to impurity management in pharmaceuticals. It will also propose a general framework for designing analytical strategies and establishing impurity acceptability thresholds based on process-related and degradation-derived impurities. Impurity profiling involves assessing contaminants to evaluate their biological safety [7]. Exposure to light, heat, free radicals, and oxygen can lead to the formation of contaminants in drug products.

Regulatory framework for impurity management

Various international and national guidelines have been established to evaluate and control impurities in pharmaceutical ingredients and products [8-10]. The ICH Q3A (R2) guideline mandates that any impurity in an API that exceeds the identification threshold must undergo structure determination studies, regardless of whether it results from the manufacturing process or degradation in stability testing [11]. Earlier versions of ICH guidelines did not emphasize impurity profiles as much. However, the updated ICH technical requirements for pharmacological registration now offer detailed instructions for verifying procedures used to examine contaminants in new drugs, residual solvents, and microbiological contaminants. A substance that was once considered pure can now be categorized into specific purity and impurity classifications, with contaminants classified as inorganic, organic, isomeric, or polymeric. The ICH guidelines on impurity control for drug substances are outlined in **Figure 1**. According to the British Pharmacopoeia (BP), impurities are divided into two categories: qualified contaminants and detectable contaminants.

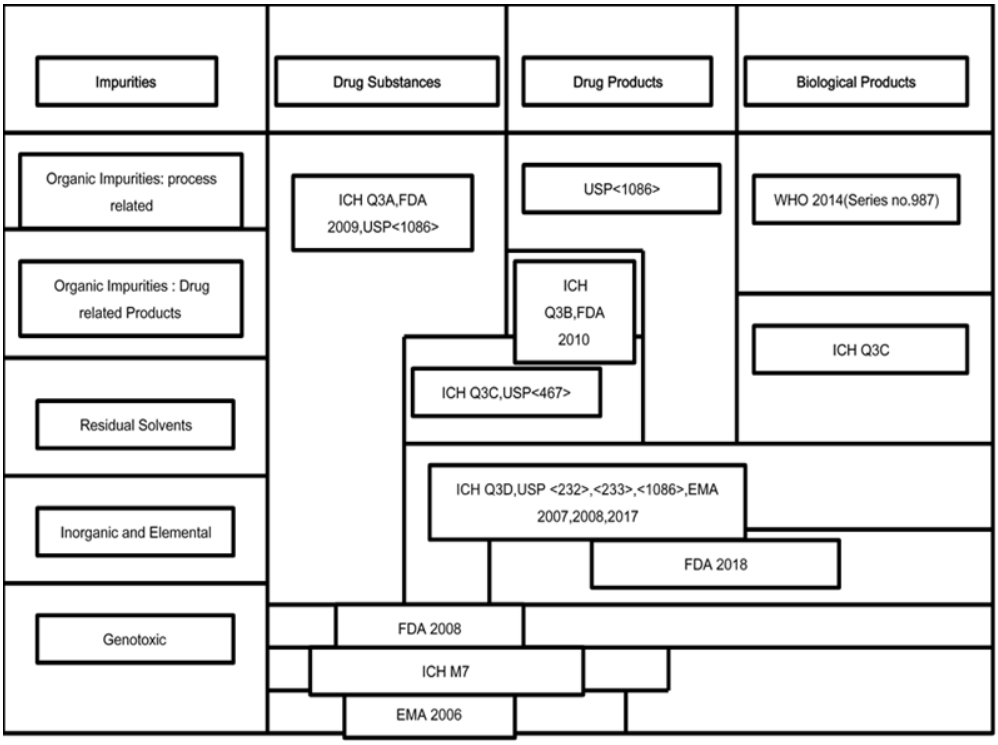


Figure 1. Guidelines for the control of contaminants in pharmaceuticals

Impurity qualifications

Modifications in key intermediates, synthesis pathways, and production scale-ups can significantly influence the impurity profile of the API. The ICH establishes guidelines to regulate and limit the introduction of new molecular entities (NMEs). The qualification process is essential for collecting and evaluating data that helps assess the

biological safety of each impurity, as illustrated in **Figure 2**. The permissible impurity levels in new pharmaceutical substances are determined based on the daily dosage administered, with higher reporting thresholds requiring scientific substantiation [12].

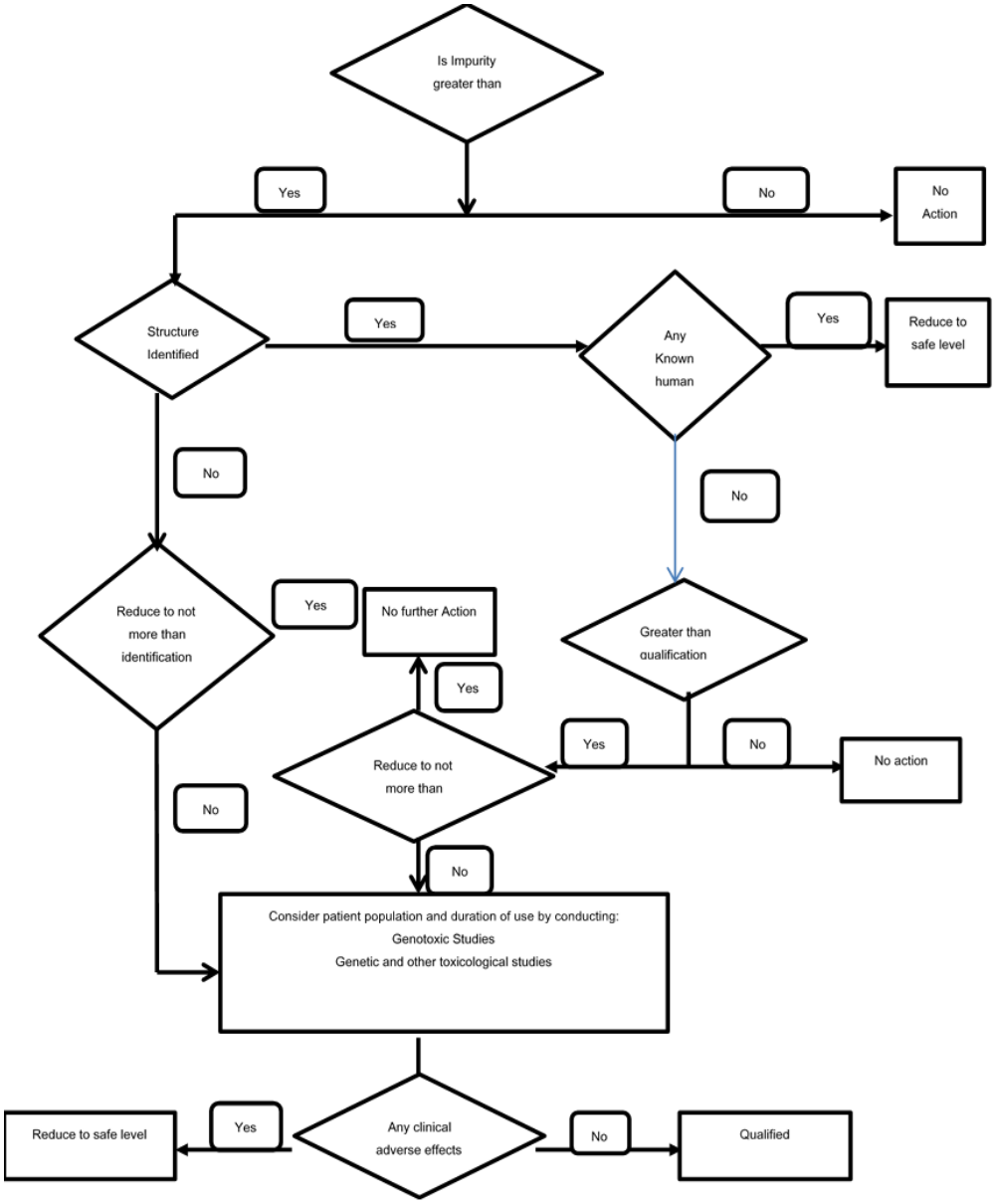


Figure 2. Identification and quantification of impurities in pharmaceuticals

Acceptability of impurities

Monitoring impurities in pharmaceutical products is essential for both safety and efficacy, as well as for ethical, financial, and competitive considerations. However, the interpretation of impurity management can vary between different sectors of the pharmaceutical industry and its associated businesses [13]. To establish uniform guidelines, the ICH collaborated with regulators and industry representatives from the EU, Japan, and the U.S. to create specific standards for contaminants in medicines [14, 15].

Origins of impurities

The identification of impurity sources that exceed the established limits is a key requirement according to ICH guidelines. Understanding where contaminants come from is fundamental for controlling their presence in pharmaceutical products. Once the source is determined, it allows for improvements in manufacturing, storage,

packaging, and prescribing practices. Pharmaceutical ingredients and final products may be affected by contaminants at multiple points in the production process. the sources of these impurities include:

- *manufacturing process contamination*: Environmental factors, such as fine particles or chemical pollutants like sulfur dioxide and hydrogen sulfide, may contaminate the production process. These substances can find their way into pharmaceutical products during the manufacturing or purification stages.
- *Crystallization-related impurities*: The pharmaceutical industry must actively manage issues like polymorphism and solvatomorphism. A polymorphic compound can crystallize in different structural arrangements, though the chemical composition remains the same. Chiral compounds, such as enantiomers, are a special case. While these substances share the same chemical composition, their spatial arrangement leads to distinct optical characteristics. Molecules with more asymmetric carbon atoms tend to contain more chiral impurities [16].
- *Formulation impurities*: After the API is synthesized, it is combined with other substances to form various dosage forms. Changes in factors like pH can impact a compound's characteristics, potentially leading to corrosion or hydrolysis of the drug, thereby introducing impurities.
- *Process-induced contaminants*: One example of an impurity is 1-(2, 6-dichlorophenyl) indolin-2-one, which forms during the manufacture of diclofenac sodium injectables. The pH levels of the formulation and sterilization processes significantly influence the generation of this impurity, as illustrated in **Figure 3a** [17].
- *Dosage form contaminants:* A notable recall in the USA involved 0.05% fluocinonide topical solution packaged in a 60 ml container. Liquid formulations are especially prone to degradation. To mitigate such risks, pharmaceutical companies perform stability and forced degradation studies during the preformulation phase to anticipate any potential issues before releasing the product. A case in point involves the precipitation of imipramine hydrochloride and sodium bisulfite when mixed in a saline solution with 5% dextrose, or the discoloration of tablets containing compounds like aminopyrine, papaverine, or theobromine [18].
- *Light-induced degradation*: Some pharmaceutical substances can become toxic when exposed to UV light. For example, ergometrine (0.2 mg/ml) completely degrades after 42 hours of sunlight exposure. Managing the exposure to light, including its intensity, wavelength, and the number of photons absorbed, is crucial in preventing such degradation.

Degradation of drug products due to impurities

As per the guidelines set by ICH, degradation products are considered impurities formed through chemical alterations in the API during production. External factors such as environmental conditions, including temperature, light exposure, humidity, and changes in pH, as well as interactions between excipients and the API, can lead to degradation during storage. It is vital to determine the chemical structures of these degradation products to understand the impact on product safety, as illustrated in **Figure 3b**. For example, hydrochlorothiazide degrades into disulfonamide, while vidagliptin contains several functional groups susceptible to degradation, forming impurities, as displayed in **Figure 3c**. Forced degradation testing is essential for assessing the impurity profile of pharmaceutical formulations. These tests help in tracking the raw materials or intermediate processes used in large-scale manufacturing, such as the synthesis of paracetamol from p-aminophenol, as displayed in **Figure 3d**. Additionally, the manufacturing process itself can introduce degradation products. A classic example is the breakdown of cephalosporin and penicillin, where the ß-lactam ring and the a-amino group in the C6/C7 side chain play a crucial role, as depicted in **Figure 3e**.

Degradation based on functional groups

- *Ester hydrolysis*: Medications with ester functional groups, especially liquid formulations, are prone to hydrolysis. Drugs like barbitol, benzylpenicillin, oxazepam, chloramphenicol, chlordiazepoxide, aspirin, benzocaine, cefotaxime, and cefpodoxime proxetil, which contains ethyl paraben, are examples of compounds that undergo ester hydrolysis, as shown in **Figure 3f** [19].
- *Oxidative degradation*: Drugs with conjugated dienes, heterocyclic aromatic rings, aldehydes, hydrocortisone, methotrexate, and adinazolam are vulnerable to oxidative degradation. The effectiveness of metal ions in promoting oxidative degradation is as follows: $Ca^{2+} > Fe^{3+} > Cu^{2+}$ [20, 21].
- *Degradation due to light exposure*: Light-induced degradation can occur when pharmaceutical products are exposed to light during manufacturing, packaging, or usage, leading to photooxidation. Medications such as

phenothiazine, riboflavin, and nifedipine are particularly susceptible to this process. For instance, ciprofloxacin eye drops (0.3%) undergo photolysis when exposed to light, resulting in the formation of ethylene diamine derivatives, as shown in **Figure 3g** [20, 22].

- *Contaminants from raw materials or intermediates*: Impurities originating from raw materials and intermediates, including isomeric impurities and incomplete reactions, are fundamental components in the creation of the final drug molecule. An example of this is the formation of a 4-trifluoromethyl impurity in 3-trifluoromethyl-α-ethylbenzhydrol due to the presence of the starting material, 3-trifluoromethyl bromobenzene [23, 24].
- *By-products*: During pharmaceutical manufacturing, side reactions such as incomplete reactions, isomerization, rearrangements, or unwanted interactions can generate by-products. Diacetylated paracetamol, a by-product, may be produced during the synthesis of paracetamol [25].
- *Inorganic contaminants*: Inorganic contaminants can enter the drug during the production of bulk pharmaceuticals, often arising from manufacturing equipment and processes. Common examples include heavy metals, persistent chemicals, and filter-related contaminants [26].
- *Catalysts, ligands, and reagents*: While rare, contaminants may originate from catalysts, ligands, and reagents. For instance, pyridinium acts as a catalyst and becomes an impurity in the production of mazipredone and pyridine [27].
- *Heavy metals*: Although water is a common solvent in manufacturing, it often contains heavy metals such as Ag, Cd, Na, Mn, and Mg. These metals can contribute to hydrolysis during drug formulation. To reduce contamination, pharmaceutical companies use demineralized water and glass-lined reactors to screen for heavy metals [28].
- *Residual solvents*: Organic volatile compounds, known as residual solvents, may remain in drugs after manufacturing. These solvents are classified based on their potential risks to human health: class I solvents are highly toxic and should be avoided, class II solvents should be used sparingly, and class III solvents pose a lower risk and are acceptable in limited amounts [29, 30].
- *Stereochemical impurities:* Stereochemistry involves the three-dimensional arrangement of atoms in a molecule, which is crucial for a drug's biological activity. Identifying contaminants related to stereochemistry is complex, especially when molecules have similar chemical structures but different spatial orientations. For instance, thalidomide exists as two isomers: the (R)-(+) form, which is calming, and the (S)-(-) form, which is carcinogenic. A comparison of the pharmacokinetics of levofloxacin (S-isomer) and ofloxacin (R-isomer) indicates no significant advantage of using a single isomer, as displayed in **Figure 3h** [31-34].
- *Water impurities*: Water used in pharmaceutical processes may contain various contaminants, including inorganic anions like chloride, phosphate, and sulfate; cations like calcium, magnesium, and sodium; organic ions such as proteins and chloramines; as well as residues from detergents, herbicides, and insecticides. Additionally, dissolved gases like nitrogen, carbon dioxide, and oxygen, as well as microbial contamination, can affect product quality [35, 36].

Elemental impurities in the pharmaceutical sector

The ICH Q3D guidelines play a pivotal role in standardizing the management of elemental impurities in pharmaceuticals. Pharmaceutical products can be contaminated by a range of elemental impurities, which may arise from excipients, catalysts, contaminants, or metals. **Table 1** outlines the sources of these impurities and their permissible limits. Elemental impurities that co-isolate with other contaminants during the pharmaceutical manufacturing process are categorized as follows:

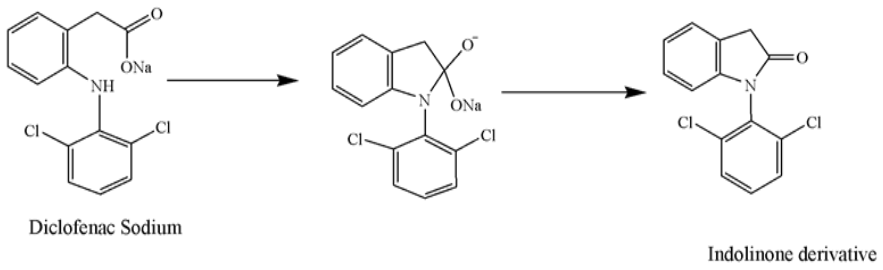
- Class 1: This class includes elemental contaminants that pose significant health risks to humans and are either entirely excluded or used only in trace amounts during drug production due to their toxic nature. Examples include lead, mercury, cadmium, and arsenic.
- Class 2: These are elemental contaminants that are toxic to humans based on the exposure route. Class 2 is further divided into two subcategories: 2A and 2B, depending on the frequency with which these contaminants appear in pharmaceutical products.
- Class 3: Elements in this category are relatively low in toxicity when ingested (with a high permissible daily exposure, PDE, of over 500 μg/day). These include substances such as antimony, tin, molybdenum, copper, lithium, chromium, and barium [37].

Table 1. Elemental impurities in drug products

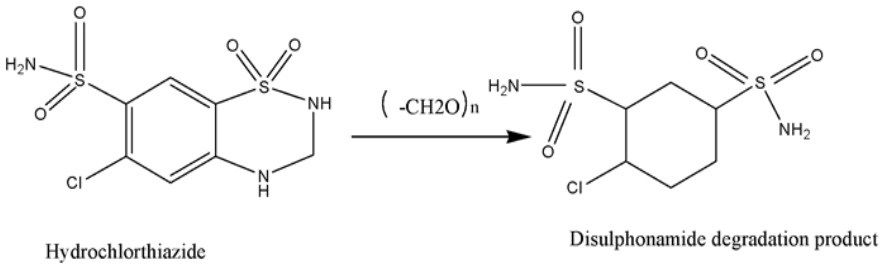
Element	Oral daily dose of PDE (mg/day)	Parenteral daily dose of PDE (mg/day)	Inhalational daily dose of PDE (mg/day)
Cadmium	5	2	2
Lead	5	5	5
Palladium	100	10	1
Inorganic arsenic	15	15	2
Nickel	200	20	5
Vanadium	100	10	1
Copper	3000	300	30

Nitrosamine contaminants

Nitrosamines have been identified in various pharmaceutical products. In collaboration with international regulatory agencies, the FDA established globally accepted standards for the maximum daily intake of nitrosamines. Following these discoveries, numerous medications containing APIs such as metformin, valsartan, losartan, and ranitidine were either recalled or removed from the market, as illustrated in **Figure 3i** [38].



a)



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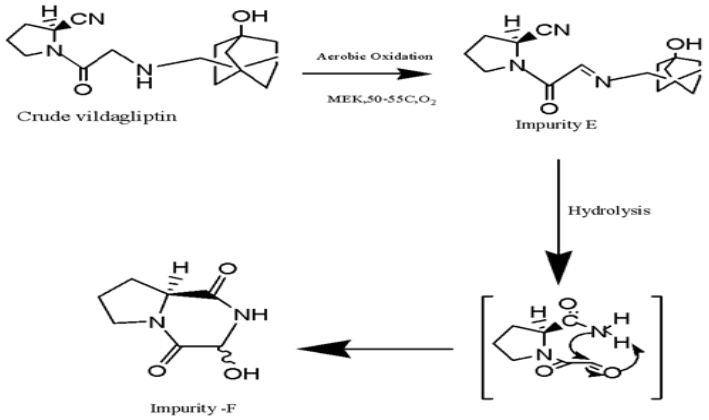
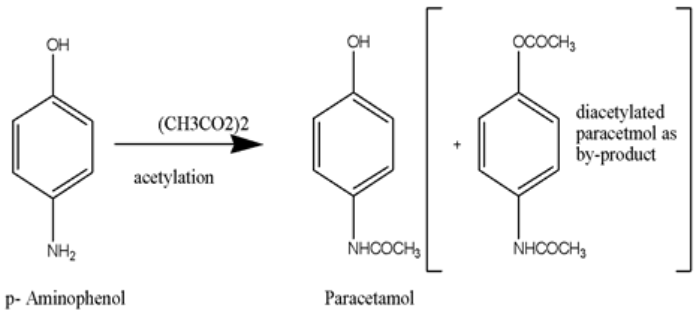
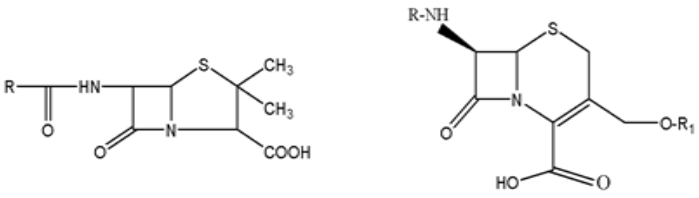


c)



p- Aminophenol Paracetamol

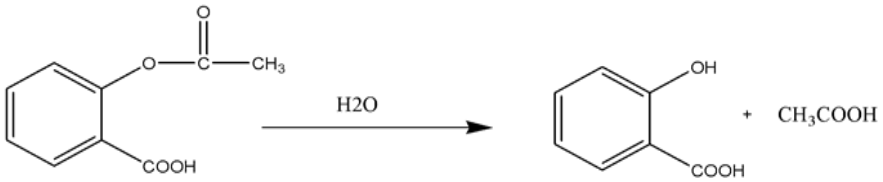
d)



A
Pencillin

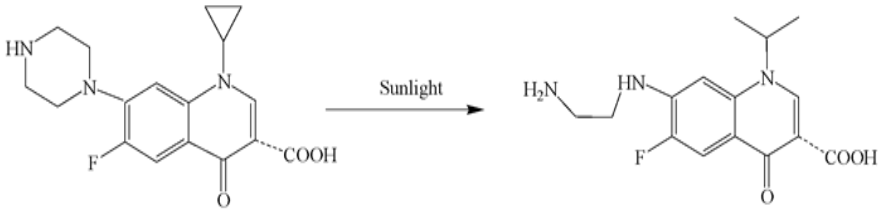
B
Cephalosporin

e)



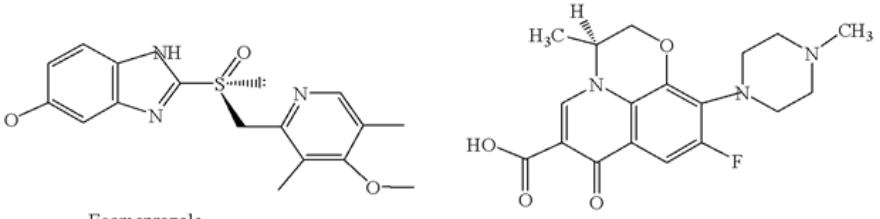
Aspirin Salicylic acid

f)

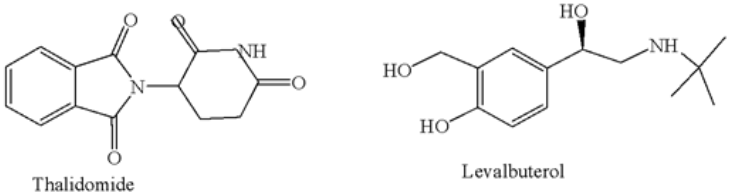


Ciprofloxacin Ethylene diamine analog

g)

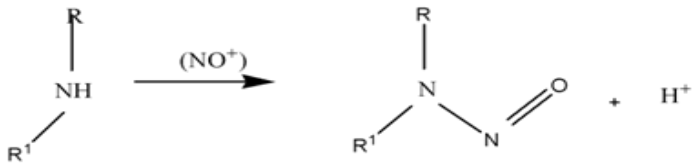


Esomeprazole Levofloxacin



Thalidomide Levalbuterol

h)



i)

Figure 3. Formation of a) indoline derivative from diclofenac sodium, b) disulphonamide degradation product, c) impurity-E and F from crude vildagliptin, d) paracetmol from P-aminophenol, e) degradation products of penicillin and cephalosporin, f) salicylic acid from aspirin, g) ethylene diamine analog, h) isomeric impurities, and i) nitrosamine impurities

- *Origins of nitro contaminants*: Nitro impurities can originate not only from external sources but also from internal processes. The body predominantly produces nitrite and nitrate in the stomach, where the latter is converted into nitrite by bacteria in the oral cavity. Studies suggest that this endogenous formation could contribute to 45% to 75% of human exposure to N-nitroso compounds [39].

It is recommended that quality risk management be used as a framework for assessing, reducing, and managing the risks related to nitrosamine contaminants in pharmaceutical products. To create an effective control strategy for each nitrosamine impurity, an appropriate acceptable intake (AI) limit must be established. This approach entails assessing APIs and finished drug products using highly sensitive and specific analytical methods.

Mutagenic impurities in pharmaceuticals

Mutagenic impurities (MIs) are substances found in pharmaceuticals that can cause genetic damage. To manage and control these impurities, global health organizations, including the FDA and EMA, have created regulatory guidelines. ICH M7 outlines methods for assessing and managing mutagenic contaminants to minimize the likelihood of cancer. Regulatory bodies typically define maximum allowable levels of mutagenic impurities, and exceeding these limits may require corrective actions, such as process adjustments or additional purification stages. Advanced testing methods, including nuclear magnetic resonance, mass spectrometry, and high-performance liquid chromatography, are used to detect and quantify these impurities. By adhering to regulatory standards and implementing stringent control practices, pharmaceutical companies aim to reduce the risks posed by mutagenic impurities [40].

Threshold of toxicological concern

The threshold of toxicological concern (TTC) provides a framework for assessing the risks associated with chemical substances, particularly when specific toxicity data is unavailable. It helps estimate an exposure level to a substance that is considered to have a low likelihood of causing adverse health effects. TTC is particularly useful when there is insufficient information on a substance's toxicity. Substances are typically categorized into different classes based on their TTC values:

- Class I: This class includes substances with a low TTC value, typically around 1.5 µg per day, and applies to chemicals with higher toxicity.
- Class II: Substances in this class have a higher TTC value, usually 30 µg per day, and are considered less toxic.

In the context of pharmaceuticals, the TTC framework is commonly used to evaluate and manage impurities in both drug substances and drug products. The ICH M7 guidelines, for instance, apply the TTC principle to manage mutagenic impurities in pharmaceuticals. A typical acceptable intake for mutagenic impurities in drugs is 1.5 µg per person per day, based on the TTC threshold, which is believed to pose a negligible risk (theoretical excess cancer risk of less than 1 in 100,000 over a lifetime). This threshold is commonly applied to long-term (over 10 years) medication use [41].

Genotoxic impurities in the pharmaceutical industry

Genotoxic Impurities in Pharmaceuticals Genotoxicity refers to the ability of a substance to disrupt a cell's genetic material, leading to mutations. Mutagenic agents, which include both chemical and physical factors, are one category of genotoxic substances. Genotoxic impurities can emerge from various stages of drug synthesis, often

as by-products or residuals of starting materials. The assessment of genotoxicity plays a crucial role in evaluating environmental toxins, chemicals, food, and feed. Even minimal exposure to genotoxic substances can lead to genetic mutations in both somatic and germ cells, potentially causing severe health consequences. Many genetic disorders are linked to mutations in specific genes like proto-oncogenes, tumor suppressor genes, or those involved in DNA repair, which can be triggered by physical and chemical agents [42].

Regulatory bodies require robust genotoxicity data for new drugs to ensure their safety and assess potential risks during the manufacturing process. Genotoxic impurities are classified into five categories:

- *Class 1*: These are known carcinogens and genotoxic agents, with evidence of their harmful nature supported by published chemical structural data.
- *Class 2*: This category includes genotoxic impurities, although they may or may not be carcinogenic. These contaminants show mutagenic properties through standard genotoxicity testing.
- *Class 3*: These impurities have uncertain genotoxic potential and differ structurally from the API.
- *Class 4*: Impurities in this group share similar functional groups or structures with the API.
- *Class 5*: These impurities do not exhibit any clear signs of genotoxicity or potential harm [43].

Regulatory approaches to controlling genotoxic impurities

- PhRMA *strategy*: This method involves categorizing compounds based on their structure, and identifying functional group patterns that have been shown to cause DNA mutations.
  - Group 1: Includes compounds with aromatic structures, such as N-hydroxyaryls, N-acylated amino-aryls, aza-aryl N-oxides, various amino-aryls, and alkylated amino-aryls, along with purines, pyrimidines, intercalators, PNAs, and PNAHs.
  - Group 2: Comprises nitro compounds, carbamates, epoxides, aldehydes, N-methylols, and N-nitrosamines, which fall under the alkyl and aryl categories.
  - Group 3: Includes heteroaromatic compounds, such as halides, haloalkenes, and alkyl esters of phosphonates and sulfonates [44].
- *ICH criteria*: According to ICH (Q3B(R2)) guidelines, impurity qualification limits are defined as a proportion of the total daily dose of the drug. These limits account for variations in pharmaceutical products and formulations [45]. Additionally, the criteria offer the possibility of applying stricter limits for substances that are highly toxic, which is particularly relevant for genotoxic impurities. The corresponding risk assessment procedures are outlined in **Table 2**.

Table 2. Risk assessment and control testing for genotoxic contaminants

Key Topic	Guidelines
Regulations for genotoxic impurity control	PhRMA position paper: Provides a detailed explanation of how to identify, assess, and manage certain drug contaminants that may cause genotoxic effects.
	EMA protocol: Focuses on the emissions of toxic or hazardous substances, introducing the concept and values related to the threshold of toxicological concern (TTC).
	FDA industry guidance: Recommends approaches for managing genotoxic and carcinogenic impurities in drug substances and products, generally following the guidelines established by the EMA.
	ICH M7 plan: A guideline for assessing and controlling DNA-reactive (mutagenic) impurities in pharmaceuticals to mitigate potential carcinogenic risks. This document is currently under development and may eventually replace the existing FDA and EMA guidelines.
Test guidelines for genotoxicity	Genotoxicity testing for pharmaceuticals (ICH S2, S2A 1996, and S2B 2007): Serves as a global reference for testing and interpreting genotoxicity data in pharmaceutical products.
	EMA (2008) guideline: Provides a framework for evaluating the genotoxicity of herbal substances and preparations, with useful methods and guidelines for interpreting potential risks.
Evaluation of genotoxic and carcinogenic chemical risks	European Commission Health and Consumer Protection Directorate (2009): Offers general methodologies and approaches for evaluating the risks of genotoxic and carcinogenic substances.

- *EU guidelines*: The CHMP (2006) provides a framework for obtaining approval for new molecular entities or for modified compounds that may introduce genotoxic impurities, particularly if they involve new synthesis routes. This guidance targets substances capable of reacting with DNA, posing a risk to its integrity. In

situations where a direct calculation for a particular substance is not feasible, the concept of the TTC is recommended. This approach calculates the daily exposure to carcinogens that would result in an extremely low cancer risk (less than 1 in a million) over a person's lifetime. For most drugs, the TTC suggests that an intake of up to 1.5 mg/day of a genotoxic impurity is deemed acceptable by the CHMP.

- *USFDA guidance*: The USFDA's recommendations align closely with those of the CHMP, offering similar approaches for addressing genotoxic impurities found in drug substances and products. These guidelines outline strategies for dealing with impurities arising from APIs and synthetic processes during clinical development or when submitting new marketing applications. The USFDA proposes that the acceptable impurity threshold be set to match a daily intake of 1.5 mg, ensuring the risk remains within safe limits. The USFDA further advises that a tiered TTC strategy is particularly suitable for shorter clinical trials, taking into account factors like variability in trial durations, assumptions from rodent lifetime studies, and challenges faced by manufacturers in detecting and controlling impurities early in the development process [46].

Methods for genotoxic impurity assessment and analysis

The assessment of genotoxic impurities is challenging due to their need to be detected at concentrations far lower than 0.01–0.03%, often requiring detection limits in the range of 1 to 5 ppm. Additionally, the reactive nature of these contaminants makes sampling more complex and necessitates extra safety measures. Currently, the most widely utilized techniques for analyzing these impurities include gas chromatography (GC) and high-performance liquid chromatography (HPLC) [47]. A visual representation of the different analytical methods employed for this purpose is shown in **Figure 4**.

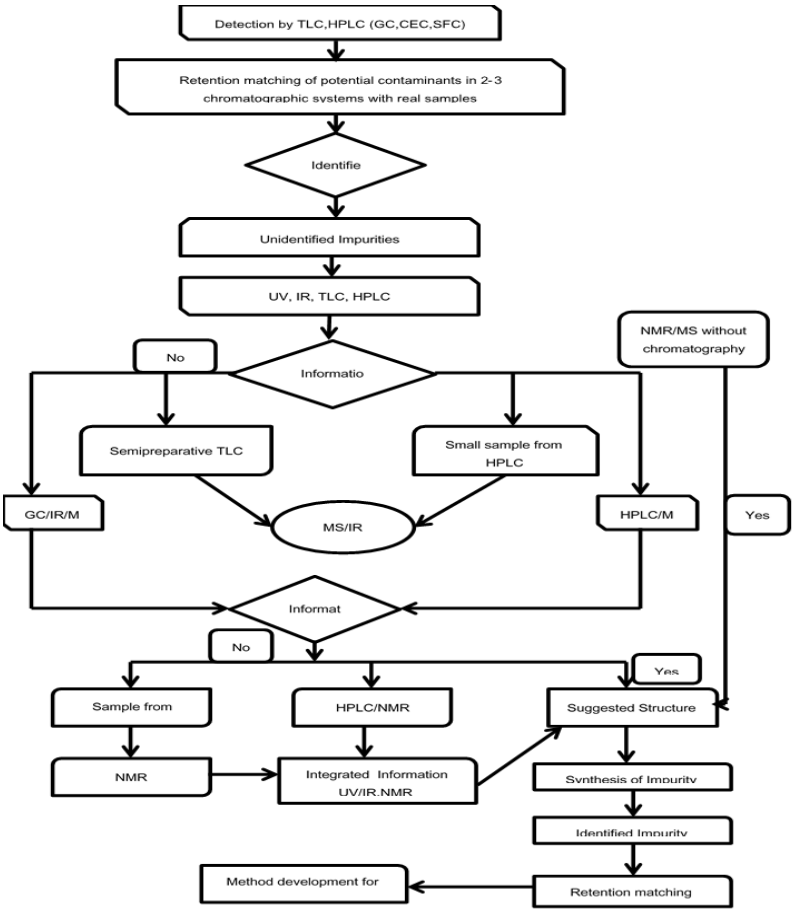


Figure 4. Analytical profiling for drug impurity

- *High-performance liquid chromatography (HPLC):* HPLC is particularly advantageous for analyzing non-volatile genotoxic substances due to its ease of use. The similarity in structure between the pollutants and the API enhances the selectivity and accuracy of HPLC. Reversed-phase HPLC is frequently employed in such

analyses. To further increase sensitivity and precision when detecting trace amounts of genotoxic impurities (GTIs), additional detectors can be incorporated. For example, the HILIC technique combined with HPLC detected five potential genotoxic impurities in dalfampridine at concentrations as low as 7.5 ppm, as illustrated in **Figure 5a**. In another case, 2,4-DNPH derivatization was applied to analyze 4-nitrobenzaldehyde in injectable formulations, converting its 4-nitrophenylhydrazone into 3-nitrophenylhydrazone, as shown in **Figure 5b** [48].

- *Gas chromatography (GC):* GC-MS and static headspace gas chromatography are regarded as highly effective for analyzing genotoxic contaminants such as halides, sulfonates, and epoxides. The headspace method is commonly employed in quality control labs for residual solvent testing, adhering strictly to ICH Q3C standards. For instance, the impurity N-nitrosodimethylamine (NDMA) was detected in valsartan tablets using GC/MS headspace analysis. NDMA, a human carcinogen, was identified as a byproduct of the production process, leading to the market withdrawal of valsartan tablets. The detection limits (LOQ and LOD) were 0.3 and 0.05, respectively, as demonstrated in **Figure 5c**.
- *Liquid chromatography and mass spectrometry (LC-MS):* LC-MS is a versatile technique used to analyze and identify the structure of impurities. The fragmentation patterns from the mass spectrometer aid in the detection of unfamiliar contaminants. This approach offers fast, efficient separation and structural clarification, which is critical in understanding the sources of impurities and reducing their levels in pharmaceuticals. The use of LC-MS/MS in research has improved efficiency, lowered analysis costs, and enabled the detection of genotoxic substances at very low concentrations. For example, LC-MS was employed to identify 2-butyl p-toluenesulfonate in naproxen, at trace levels of approximately 1 ppm, as shown in **Figure 5d** [49].
- *Inductively coupled plasma mass spectrometry (ICP-MS) with optical emission spectroscopy (ICP-OES): I*CP-MS coupled with ICP-OES is a powerful technique for detecting metal contaminants that may pose a genotoxic risk. Elemental impurities can be analyzed either directly or after sample preparation by dissolving the sample in an aqueous or organic solvent [50, 51].
- *Nuclear magnetic resonance spectroscopy (NMR):* NMR spectroscopy is particularly valuable for examining the stereochemistry and bonding of molecules. It is essential for the structural analysis of genotoxic impurities and degradants at low concentrations. NMR is non-destructive, allowing for the study of pollutants at minute levels without altering the sample's integrity.

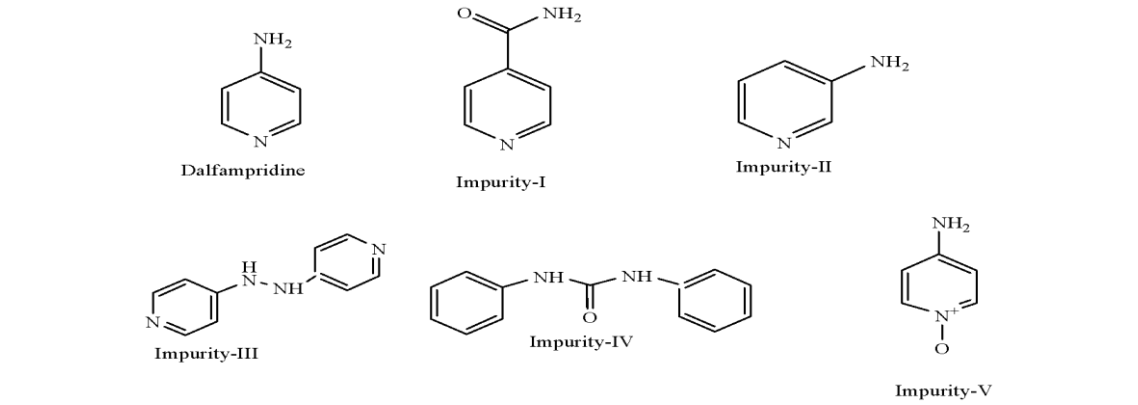
Pharmaceutical recalls due to carcinogenic and genotoxic contaminants

In recent years, numerous pharmaceutical products have been withdrawn from the market due to contamination by nitrosamines, which are both carcinogenic and genotoxic. These substances have been linked to cancer in animal models. Over the past two years, more than 1400 product lots have been recalled after their nitrosamine levels surpassed the established daily limits. The presence of these impurities in APIs such as valsartan, irbesartan, losartan, metformin, ranitidine, and nizatidine led to their removal from the market or discontinuation of distribution [52].

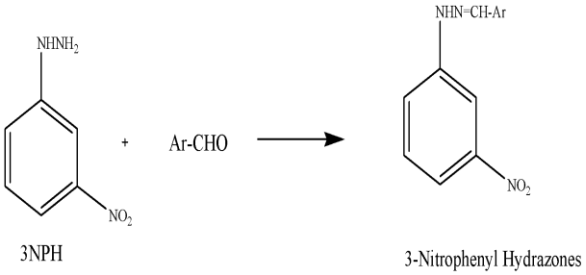
- *Valsartan*: The 2018 recall of valsartan, a widely used angiotensin II receptor blocker (ARB), marked one of the earliest cases. Novartis, the developer of Diovan (valsartan), found that both generic and brand-name versions of ARBs contained nitrosamine impurities like NDMA and NDEA [53].
- *Irbesartan*: In October 2021, Lupin Pharmaceuticals recalled several batches of irbesartan and the combination of irbesartan with hydrochlorothiazide after they were found to contain high levels of N-nitroso irbesartan. The brand-name version of the drug, Avapro, is marketed by Sanofi, with generic alternatives available for over a decade.
- *Nizatidine*: Nizatidine, used for treating gastric reflux, duodenal ulcers, and esophagitis, was recalled in January 2020 by Mylan due to NDMA contamination. In April of the same year, Amneal Pharmaceuticals also voluntarily recalled large quantities of nizatidine oral solution.
- *Quinapril*: Quinapril, a blood pressure medication, was recalled by Lupin Pharmaceuticals in December 2022 over concerns related to nitrosamine contamination. In March 2022, Pfizer also recalled five batches of quinapril pills due to high levels of the nitrosamine N-nitroso-quinapril, as well as significant quantities of quinapril/hydrochlorothiazide tablets under the Accuretic brand.
- *Rifampin and rifapentine*: Rifampin, used in combination with other drugs for tuberculosis treatment, was found to contain nitrosamine impurities in 2020. The FDA discovered multiple batches of rifampin and

rifapentine with elevated levels of 1-methyl-4-nitrosopiperazine and 1-cyclopentyl-4-nitrosopiperazine, leading to regulatory actions and efforts to address potential shortages, as shown in **Figure 5e**.

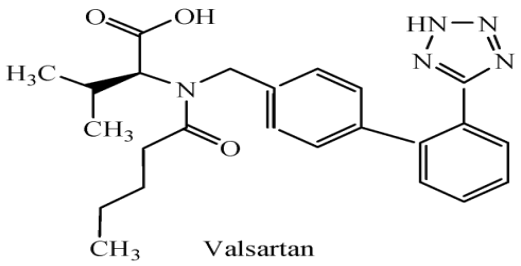
- *Sitagliptin*: Merck and Co. disclosed in August that their sitagliptin-containing medications (Januvia, Janumet, and Steglujan) contained nitrosamine impurities. The FDA indicated that if the nitroso-STG-19 levels in sitagliptin exceeded the acceptable threshold, the drug might be temporarily removed from circulation.
- *Varenicline*: In June 2021, Pfizer halted the export of Chantix due to the discovery of the nitrosamine N-nitroso-varenicline. Later that year, the company expanded the recall, and by February 2022, a federal judge dismissed a lawsuit concerning the contamination, as shown in **Figure 5f**.
- *Losartan*: Losartan, an angiotensin II receptor blocker, was affected by nitrosamine contamination in 2019, leading to a large-scale recall by Torrent Pharmaceuticals. The issue was linked to the API from Hetero Labs Ltd., as shown in **Figure 5g**.
- *Metformin*: In 2020, the FDA detected NDMA in metformin, a widely used medication for managing type 2 diabetes, which led to recalls of affected lots.



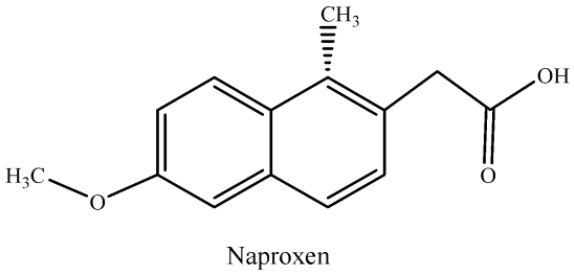
a)



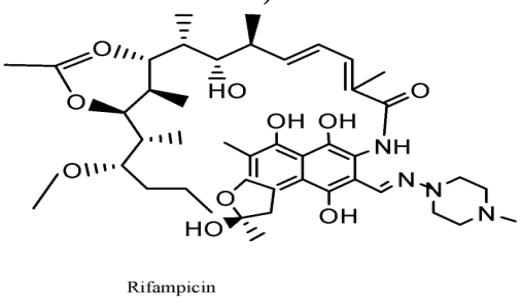
b)



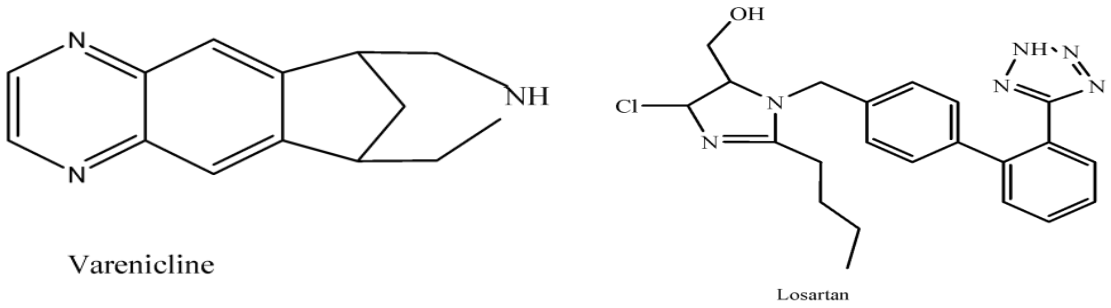
c)



d)



e)



f) g)

Figure 5. Structure of a) dalfampridine and its impurities, b) 3-nitrophenylhydrazone, c) valsartan, d) naproxen, e) rifampicin, f) varenicline, g) losartan

- *Ranitidine*: This medication reduces stomach acid production. In 2019, certain batches of the over-the-counter heartburn treatment ranitidine were discovered to contain elevated levels of nitrosamines, including NDMA. Following laboratory testing by Valisure, which detected NDMA contamination, the FDA later directed manufacturers to remove ranitidine from distribution.

Conclusion

This review article examines contaminants present in drug substances and pharmaceutical products, offering valuable insights into different impurity types, their classification, sources, and methods for their identification, isolation, and characterization. It also highlights the identification and categorization of genotoxic impurities. The study's findings emphasize that the implementation of precise, sensitive, and quantitative analytical techniques for detecting and measuring GTIs will contribute to the establishment of a regulatory framework that enhances drug safety for both pharmaceutical products and individuals. Additionally, several control strategies have been compiled to facilitate the regulation of GTIs in the early stages of drug development. A comprehensive discussion on impurity profiling and related topics, as presented in this paper, may be of broad and significant interest.

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