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Review Article

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Pharmaceutical Impurities and Their Regulatory Aspects with a Special Focus on Genotoxic Impurities

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ABSTRACT

Drug impurity profile studies have changed significantly during the past ten years, as demonstrated by pharmacopeia and regulatory standards. This review article aims to give viewers a thorough understanding of the numerous facts of impurity profiling about regulatory criteria. Comprehensive information on residual solvents, water impurities, elemental impurities, carcinogenicity, and an overview of the most critical components of genotoxic contaminants. To ensure the drug products fulfill the minimal acceptable quality standards many pharmacopoeias have created monographs. ICH, EMEA, USFDA, and European Pharmacopeia recommendations are just a few of the regulatory bodies that offer rules to reduce the amount of contaminants in medications. To prevent impurities, industry, research, and development sectors widely employ different spectrum analyses including HPLC, LC/MS, and GC/MS. It involves the recall of medications because of these contaminants. The significance of understanding genotoxic impurities as a crucial element of the impurity profile in medications is emphasized in this abstract.

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

INTRODUCTION

The pharmaceutical sector has been very interested in drug impurity profiling over the past ten years. Even minute concentrations of these unwanted substances have the potential to jeopardize the efficacy and safety of pharmaceutical products. Several pharmacopeias have developed monographs to ensure that drug substances and drug products meet minimum acceptable quality standards for consumers, including the British Pharmacopoeia, Indian Pharmacopoeia, European Pharmacopoeia, and United States Pharmacopoeia (USP) [1-3]. Even if the impurity has superior medicinal effects or toxicological qualities, the presence of it in the drug material damages the drug's purity [4]. ICH is defined as an impurity in a pharmacological substance or any ingredient that is a part of the drug, not the substance that is chemically defined as a drug that has an impact on the pharmacological compounds or active ingredients purity. Sources of impurities must be appropriately categorized to develop and apply various regulatory standards and management techniques. The contaminant characteristics of medications are becoming increasingly and more significant as medication safety garners media and public interest. Regulations can be obtained from US and foreign authorities and this topic is covered in several recent books and journal reviews [5, 6].

This review article aims to give viewers a thorough understanding of the numerous facts of impurity profiling about regulatory criteria. Comprehensive information on residual solvents, water impurities, elemental impurities, carcinogenicity, and an overview of the most critical components of genotoxic contaminants.

RESULTS AND DISCUSSION

Overview of impurities in the pharmaceutical sector

Impurity profiling of APIs has begun to gain more popularity since it has been shown that impurities in APIs can impair the quality and safety of medicinal products. Impurity detection, isolation, and measurement are crucial steps in both medication development and regulatory evaluation. Pharmaceutical contaminants are chemicals that exist together with APIs or may emerge during product aging or API manufacturing. Even trace levels of these contaminants can affect a drug's effectiveness and safety. Generally a rise in impurity research, there are still several issues with the creation of techniques for identifying substance degradation and processes. The main objective of this study is to give a summary of the most significant international regulatory standards that are currently in force regarding the management of impurities in pharmaceutical goods. Following this, a general plan for designing an analytical approach and acceptability standards for impurities related to process and degradation can be suggested. The method of assessing data to determine the biological safety of specific contaminants is known as impurity profiling [7]. Contaminants may develop in drug products as a result of exposure to sunlight, heat, free radicals, and air.

Essential regulations about impurity management

Numerous international and local recommendations and directives Guidelines have been issued for evaluating and controlling impurities in pharmaceutical ingredients and medications [8-10]. ICH Q3A (R2) requires that all contaminants in API exceeding the identification threshold undergo studies to determine their structures, regardless of their presence in batches produced using the recommended process or in degradation products from stability studies [11]. The impurity profile of the medications was not heavily stressed in the past editions. The ICH guideline has been issued for technical requirements for the registration of pharmacological for human use. It provides instructions for verifying the accuracy of procedures used to examine contaminants in newly developed medications, products, leftover solvents, and microbiological contamination. If a substance that was previously thought to be pure can now be classified into new purity and impurity categories, then components that are inorganic, organic, isomeric, or polymeric are regarded as impurities. Various guidelines specified by ICH for the control of impurities in drug substances are given in **Figure 1**. As per BP, impurities are classified into two subtypes: qualified contaminants and detectable contaminants.

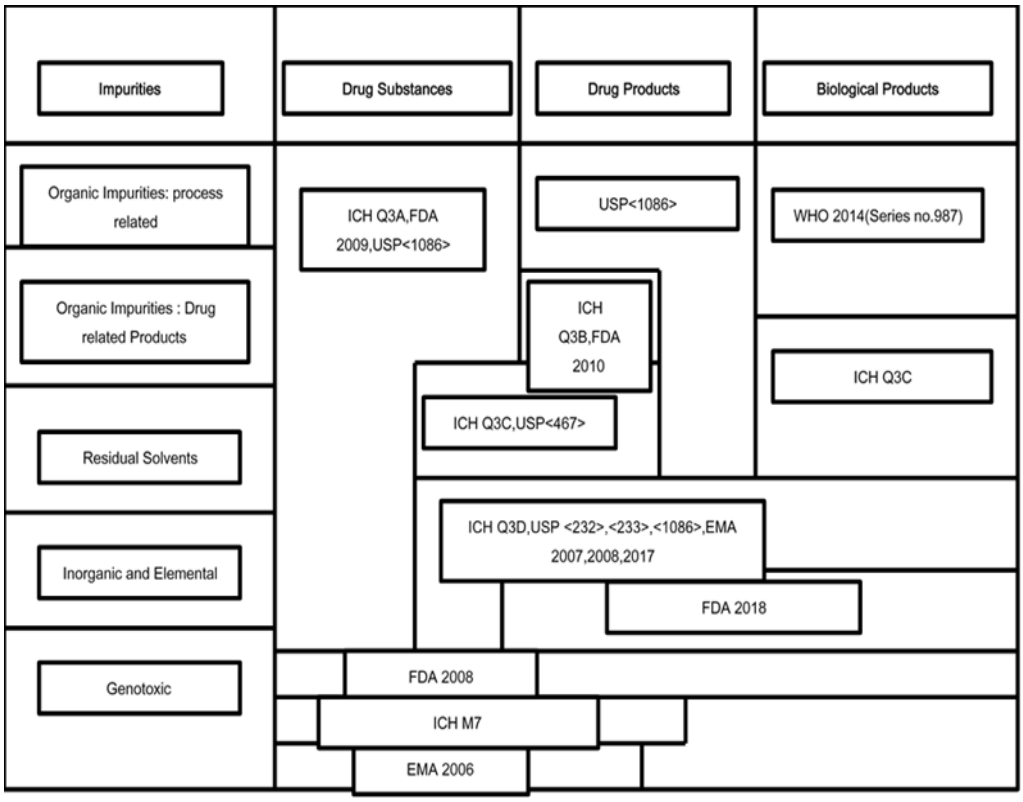


Figure 1. Guidelines for the control of contaminants in pharmaceuticals

Impurity qualifications

Changes in critical intermediates, synthesis routes, and scale-ups can all affect the impurity profile of the medicinal ingredient. The ICH categorizes and restricts new molecular entity restrictions. The qualification procedure aids in gathering and assessing information that determines each impurity's biological safety as mentioned below in **Figure 2**. The impurity limits in novel pharmacological substances depend on the daily dosage of the pharmacological substance delivered, higher reporting requirements have to be supported by science [12].

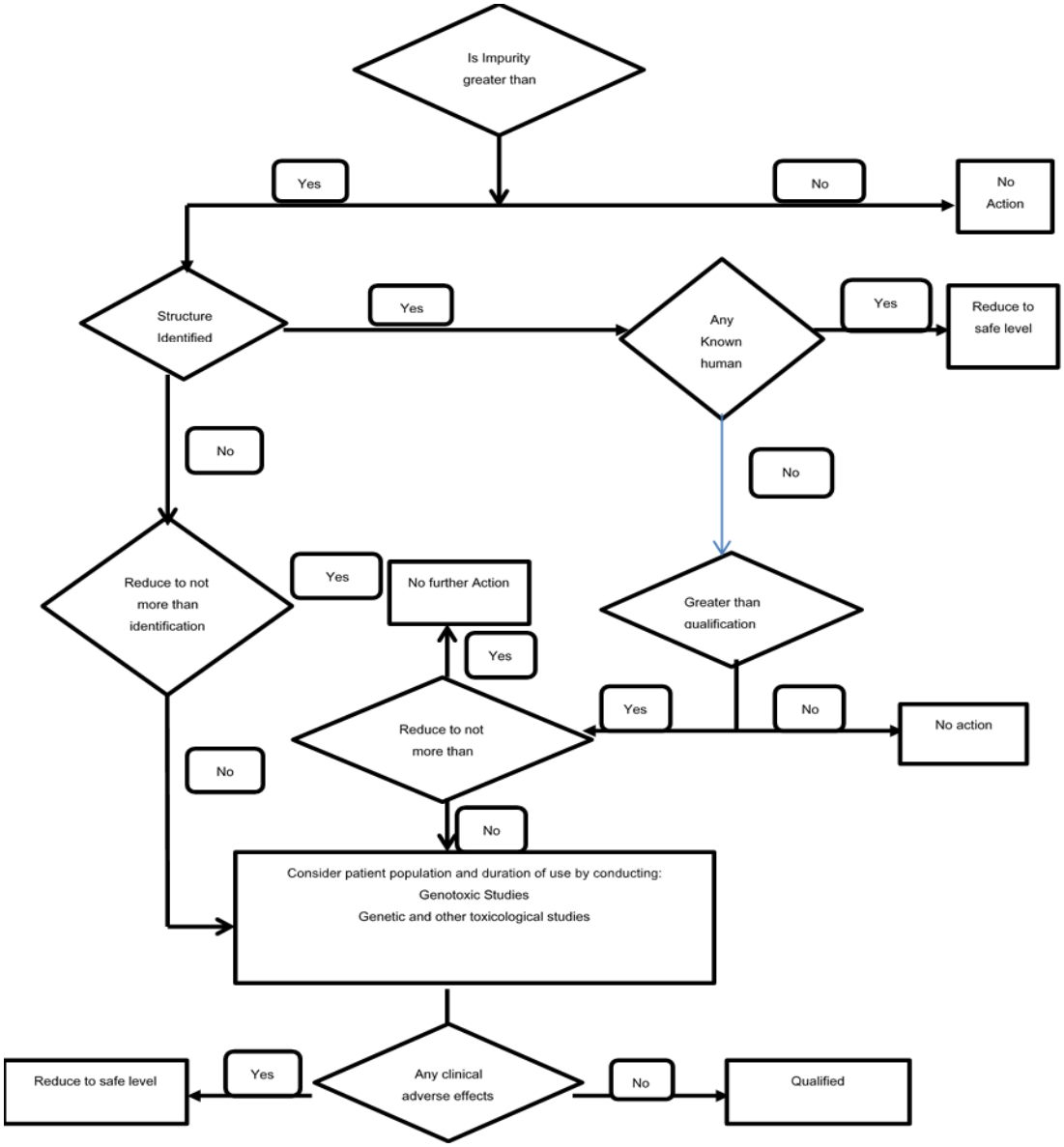


Figure 2. Identification and quantification of impurities in pharmaceuticals

Acceptability criteria for impurity

Monitoring contaminants in medicinal products is necessary for safety and effectiveness concerns, as well as ethical, financial, and competitive reasons. But even among individuals in the pharmaceutical sciences and business, managing and monitoring contaminants might imply various things [13]. EU, Japan, and the United States collaborated with regulators and industry representatives to produce the ICH guideline for contaminants in medicines [14, 15].

Sources of impurities

The identification of the source of contaminants in drugs that exceed the authentication limit is mandatory, as stated explicitly in the ICH standards. Contaminant source evaluation is the cornerstone of pharmaceutical

contaminant control. By identifying the source of the impurity, the manufacturing, prescribing, packing, and storage conditions of the medication can be enhanced. Pharmaceutical dosage forms and prescription items may contain impurities from a range of sources at different stages of manufacture. Pharmacological substances can also contain impurities.

- *Pollution caused during the manufacturing process:* Industrial areas are contaminated by fine particles and substances including sulfur dioxide, and hydrogen sulfide. When pharmaceuticals are manufactured or purified, these impurities find their way into the finished products.
- *Impurities associated with crystallization:* The pharmaceutical industry is expected to actively participate in polymorphism and solvatomorphism following the regulations set forth by the regulatory bodies. A crystal system is said to be polymorphic when it can exist in several crystal packing patterns with the same elemental composition. Chiral substances are often referred to as enantiomers. These optical isomers have identical chemical structures, yet because of their distinct spatial layout, they have different optical rotations. It is important to remember that the more asymmetric carbon there is in a molecule, the more chiral impurities there are in the atoms [16].
- *Impurities related to formulation:* The next step after producing an API is to mix it with additives to create a variety of dosage forms, including liquids, pills in pill form, fine particles, semi-solids, and other novel drug delivery modalities. A pH shift, for example, might alter a compound's lubricity through corrosion and speed up its hydrolysis.
- *Associated with a procedure:* 1-(2, 6-dichlorophenyl) indolin-2-one is a contaminant that is produced during the manufacturing of diclofenac sodium in its injectable form. The pH of the formulation and the sterilization parameters affect the production of this contaminant as shown in **Figure 3a** [17].
- *Associated with dosage form:* For example, the active ingredient in 0.05% fluocinonide topical solution in a 60 ml container was recalled in the USA. Liquid dosage formulations have a higher vulnerability to degradation. Pharmaceutical companies carry out preformulation research, including stability and forced degradation trials, to anticipate such degradative possibilities before releasing any medicine onto the market. In a saline solution with 5% dextrose, imipramine hydrochloride, and sodium bisulfite precipitate. When pills contain aminopyrine, papaverine, and theobromine, causes discoloration of the tablets [18].
- *UV light:* Many pharmaceutical substances become poisonous when exposed to light. Ergometrine (0.2 mg/ml) completely degrades after 42 hours in the sun, according to a study. It is essential to regulate the wavelength, intensity, and amount of photons absorbed by the light.

Degradation of drug products due to the presence of impurities

According to the ICH rules, degradation products are pollutants resulting from chemical changes made to the medication ingredient during the production processes. Various environmental factors, including light, temperature, humidity, and pH variations, as well as the interaction between any excipient components and the API, when storing the medication may lead to degradation. Therefore, it is necessary to ascertain the chemical structures of these products as shown in the **Figure 3b**. Disulfonamide for example, is the component element that hydrochlorothiazide breaks down. For instance, vidagliptin has several functional groups that can break down and produce contaminants as shown in **Figure 3c**. Forced degradation studies are considered a powerful tool in this context, providing an acceptable contaminant profile for the drug formula. This test can be used as a raw material for a single producer or as an intermediate step to demonstrate the process of producing paracetamol from the intermediate p-aminophenol, as shown in **Figure 3d**. The final product may break down and produce contaminants during the manufacturing of medications in large quantities. One well-known example of degradation products is the degradation of cephalosporin and penicillin. The ß-lactam ring and a-amino group in the C6/C7 side chain play a critical role in their breakdown as shown in **Figure 3e**.

Functional group-specific degradation

- *Ester hydrolysis*: Hydrolysis is a common occurrence with medications of the ester type, especially in liquid dosage forms. Barbitol, benzylpenicillin, oxazepam, chloramphenicol, chlordiazepoxide, aspirin, benzocaine, cefotaxime, echothiophate from cocaine and ethyl paraben-containing cefpodoxime proxetil are a few examples as shown in **Figure 3f** [19].
- *Oxidative degradation*: In addition to conjugated dienes, heterocyclic aromatic rings, aldehydes, hydrocortisone, methotrexate, and adinazolam are also included in this list. The following is the sequence of metal efficacy in AEB breakdown: $Ca^{2+} > Fe^{3+} > Cu^{2+}$ [20, 21].

- *Light-induced cleavage*: If pharmaceutical products are exposed to light during production, packaging, or ordinary usage, they may experience photooxidation. Phenothiazine, riboflavin, and nifedipine are medications that are vulnerable to photooxidation. Photolysis happens when ciprofloxacin eye drops (0.3%) are exposed to light. Ethylene diamine analogs of ciprofloxacin are produced as a result of this shown in **Figure 3g** [20, 22].
- *Starting materials or intermediate contaminants:* Starting materials, which are primarily isomeric impurities and intermediates, which are incomplete reactions or reagent excesses, are the chemical building blocks that combine to generate the final form of a pharmaceutical molecule. The presence of 3-trifluoromethyl bromobenzene, the synthesis starting material, causes an isomeric 4-trifluoromethyl impurity to exist in 3-trifluoromethyl-α-ethylbenzhydrol [23, 24].
- *By-products*: In organic chemistry, Byproduct generation can occur from a variety of side reactions, such as incomplete reactions, rearrangements, dimerization, overreactions, isomerization, and unwanted interactions involving initial components. For example, diacetylated paracetamol may arise as a by-product during the manufacturing process of paracetamol [25].
- *Inorganic contaminants*: In the formulation of bulk medications, these are obtained during the manufacturing process. These include pollutants such as heavy metals, persistent chemicals, and other materials, like filters [26].
- *Catalysts, ligands, and reagents:* Contaminants of this type are extremely rare. Pyridinium acts as a catalyst since it turns into an impurity when mazipredone and pyridine are produced [27].
- *Heavy metals:* Even though water is used in most manufacturing processes, heavy metals are unfortunately present in significant amounts in it. The addition of Ag, Cd, Na, Mn, and Mg to the reaction media may cause drug hydrolysis. Pharmaceutical items are screened for heavy metal contamination using demineralized water and glass-lined reactors [28].
- *Lingering solvents:* Organic volatile compounds that are utilized in manufacturing or produced during production are known as residual solvents. Residual solvents are classified into three categories based on the potential harm to human health. Class I solvents are either completely avoided or used very sparingly for making excipients and medication materials because of their unacceptable level of toxicity. Class II Solvents should only be used sparingly in medicinal applications. Class III Solvents did not pose a significant risk to human health since they are less hazardous and have a lower risk than class I or class II solvents [29, 30].
- *Impurities linked to stereochemistry:* Stereochemistry is the study of a molecule's three dimensions; the spatial arrangement of a drug's atoms determines how well it functions in a biological system. Finding molecules related to stereochemistry that have comparable chemical structures but different spatial orientations and compounds that might be viewed as contaminants in the APIs represents a significant undertaking [31, 32]. Two isomers of thalidomide exist. The calming and hypnotic properties of (R)-(+) thalidomide contrast with the carcinogenic activity of (s)-(-) thalidomide. A comparison of the pharmacokinetic characteristics of levofloxacin (Sisomeric form) and ofloxacin (R-isomeric form) shows that there are no advantages to using a single isomer drug's active ingredient and its structure as previously indicated as shown in **Figure 3h** [33, 34].
- *Impurities from water*: Water pollution may have an impact on the quality of results in a pharmaceutical setting. Inorganic anions like chloride, phosphates, sulfates, and nitrates; cations like calcium, magnesium, and sodium of inorganic nature may contaminate water; organic ions like proteins, chloramines, and residues from detergents, insecticides, and herbicides; dissolved gases including carbon dioxide, nitrogen, and oxygen; microbes viz., algae, lead contamination of water [35, 36].

Elemental impurities in the pharmaceutical industry

The ICH Q3D is a crucial set of guidelines for harmonizing elemental impurity management. Pharmaceutical products may contain a variety of elemental impurities, including excipients, catalysts, contaminants, and some metals. The sources of elemental impurities and the acceptable limits are given in **Table 1**. The co-isolated impurities with other elemental impurities in pharmaceutical process materials, They are

- Class 1: The elemental contaminants that are hazardous to humans and are either not used at all or very little in the production of medications. Because of their harmful effects on humans. It includes lead, mercury, cadmium, and arsenic.

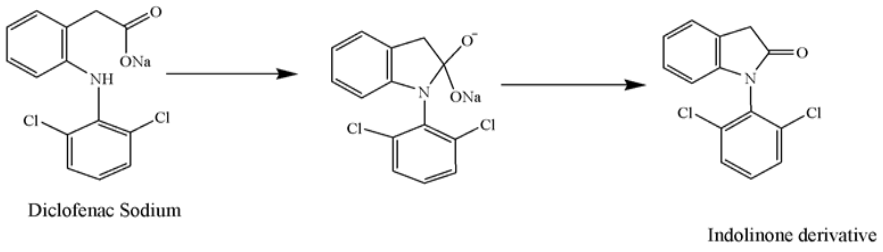
- Class 2: Human toxicants that are reliant on a route are the elemental contaminants that fall under this class. They are further separated into two i.e. 2A and 2B, according to how often they appear in pharmaceutical medication products.
- Class 3: The elemental contaminants in this class are not very hazardous when taken orally (high PDE of more than 500 μg/day). It includes antimony, tin, molybdenum, copper, lithium, chromium, and barium [37].

Table 1. Elemental impurities in drug products

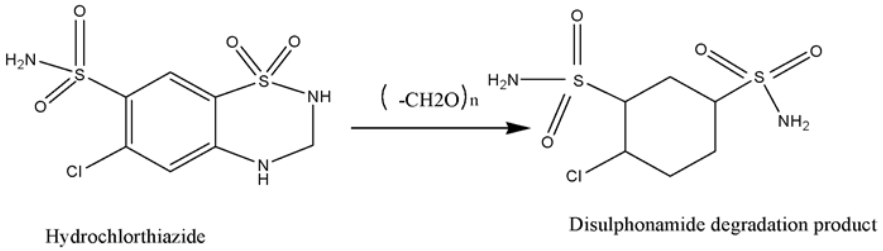
Element	Oral Daily Dose PDE (mg/day)	Parenteral Daily Dose PDE (mg/day)	Inhalational Daily Dose PDE (mg/day)
Cadmium	5	2	2
Lead	5	5	5
Palladium	100	10	1
Inorganic arsenic	15	15	2
Nickel	200	20	5
Vanadium	100	10	1
Copper	3000	300	30

Nitrosamine impurities

Nitrosamines were detected in a variety of drug goods, With the help of foreign regulatory colleagues the FDA has set globally recognized guidelines for the acceptable daily consumption of nitrosamines. As a result of the finding, many pharmaceutical products containing the APIs metformin, valsartan, losartan, and ranitidine, were taken off the market or had their recalls as shown in **Figure 3i** [38].



a)



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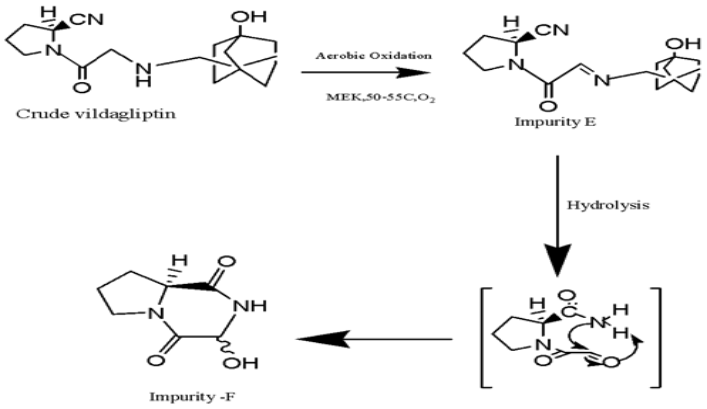

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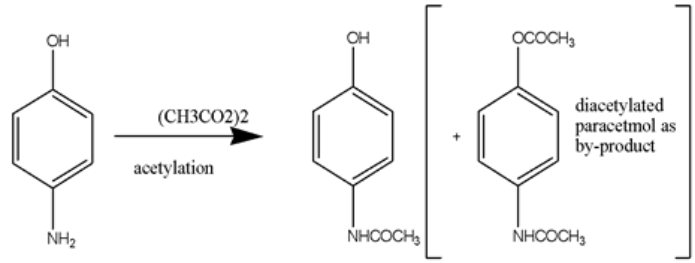
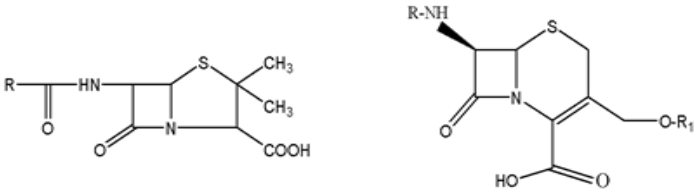


p- Aminophenol Paracetamol

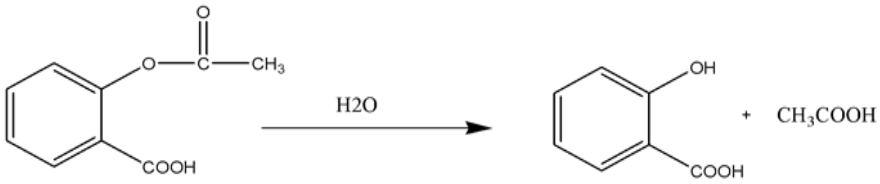
d)



A
Pencillin

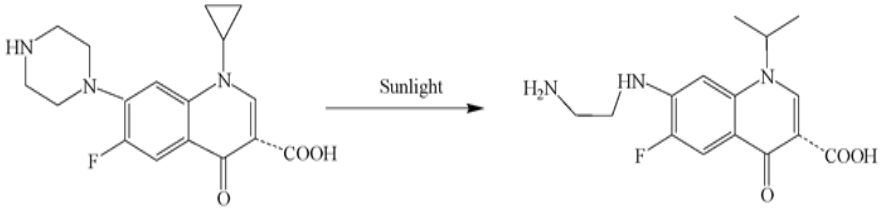
B
Cephalosporin

e)



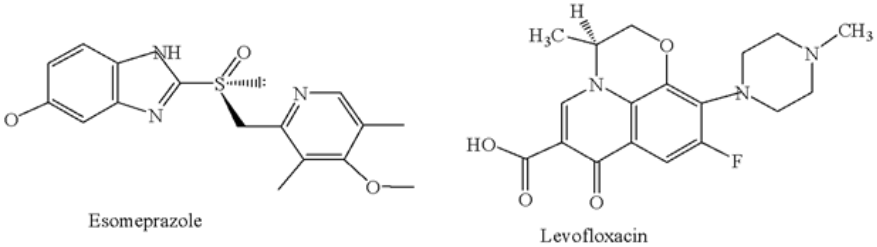
Aspirin Salicylic acid

f)

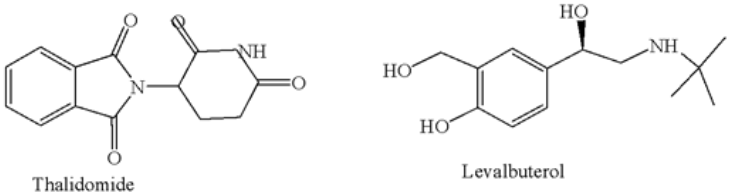


Ciprofloxacin Ethylene diamine analog

g)

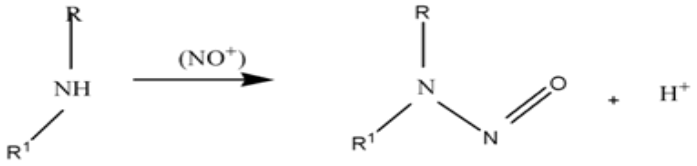


Esomeprazole Levofloxacin



Thalidomide Levalbuterol

h)



i)

Figure 3. Formation of a) Indoline derivative from Diclofenac Sodium, b) Disulphonamide degradation product, c) Impurity-E & F from Crude Vildagliptin, d) Paracetamol from P-Aminophenol, e) Degradation Products of Penicillin & Cephalosporin, f) Salicylic acid from Aspirin, g) Ethylene diamine analog, h) isomeric impurities, and i) Nitrosamine impurities

- *Sources of nitro impurities:* In addition to being exposed to nitrosamines exogenously, these chemicals can also be created endogenously. The endogenous production of nitrite and nitrate is mostly carried out in the stomach from the latter, which is changed into nitrite by oral cavity bacteria. Reports indicate that it might account for anywhere from 45-75% of human exposure to N-nitroso compounds [39].

It is advised that quality risk management serves as a guide for the evaluation, mitigation, and control of hazards associated with nitrosamine contaminants in human medicines. An AI limit is required to develop a suitable control approach for individual nitrosamine impurities in human medicinal products. This strategy involves evaluating APIs and drug products using analytical techniques that are sensitive enough and sufficiently specific.

Mutagenic impurities in pharmaceuticals

In the pharmaceutical industry, materials that can cause genetic abnormalities are referred to as mutagenic impurities or MIs. Therefore, regulatory agencies such as the FDA, EMA, and other global health authorities have established guidelines to control and limit the presence of mutagenic impurities in pharmaceutical products. ICH M7 describes how to evaluate and manage mutagenic contaminants in drugs to reduce the risk of cancer. Regulatory agencies typically specify acceptable limits for mutagenic impurities in pharmaceuticals. If a mutagenic impurity exceeds the permitted limit, corrective actions may be required, such as process optimization or additional purification steps. To identify and measure mutagenic contaminants, sophisticated analytical methods like nuclear magnetic resonance, mass spectrometry, and high-performance liquid chromatography are utilized [40]. By adhering to regulatory guidelines and implementing effective control strategies, pharmaceutical companies aim to minimize the potential risks associated with mutagenic impurities.

Threshold of toxicological concern

The threshold of toxicological concern is a risk assessment approach used in the evaluation of chemical substances, particularly in the absence of specific toxicity data. It is employed to estimate a level of exposure to a substance below which there is a low probability of adverse effects on human health. The TTC concept is typically used when there is a lack of specific toxicity data for a particular substance. TTC values are often categorized into different classes of chemicals, such as:

- Class I: Substances with a lower TTC value i.e., 1.5 µg per day usually applied to more toxic substances.
- Class II: Substances with a higher TTC value i.e., 30 µg per day applied to substances with lower toxicity.

In the pharmaceutical industry, the TTC concept is frequently used to assess and control impurities in drug substances and drug products. The ICH M7 guideline applies the TTC concept to assess and control mutagenic impurities in pharmaceuticals. In general, most pharmaceuticals can use this TTC-based acceptable intake of 1.5 µg of mutagenic impurity per person per day as a default to derive an acceptable limit for control, as it is thought to be associated with a negligible risk (theoretical excess cancer risk of < 1 in 100,000 over a lifetime of exposure). This method is typically applied to mutagenic contaminants found in medications intended for long-term (> 10 years) usage [41].

Genotoxic impurities in the pharmaceutical industry

Genotoxicity is the term used to describe a harmful consequence that compromises a cell's integrity and damages its genetic material. Mutagenic substances include radiation as well as chemical agents. Genotoxic impurities can enter a drug's synthesis via a variety of sources, mostly as starting materials and their impurities. Genotoxicity data is the cornerstone used to evaluate the risk of naturally existing environmental toxins in chemicals, food, and

feed. Even low amounts of exposure can cause genetic alterations in somatic and germ cells, leading to detrimental health effects. Many genetic diseases result from mutations in proto-oncogenes, tumor suppressor genes, or DNA damage response genes induced by various agents, such as physical and chemical factors [42]. International regulatory organizations require reliable data on the genotoxicity of new drugs to provide evidence of the safety review of the product and its manufacturing process. These are classified into five types:

- *Class 1*: Impurities that are known to cause cancer and to be genotoxic. The impurity's genotoxic nature is illustrated by published chemical structural data.
- *Class 2:* Recognized genotoxic impurities that may or may not cause cancer. This category comprises contaminants that are mutagenic by testing in traditional genotoxicity assays.
- *Class 3:* Impurities are substances that have an ambiguous genotoxic capability and a distinct structure not connected to the active pharmaceutical ingredient's structure.
- *Class 4:* Impurities consist of substances that either have a similar functional group as the active pharmaceutical ingredient or are related to the API through a similar structure.
- *Class 5:* Impurities have the absence of any warning signs or genotoxic potential signals [43].

Regulatory guidelines on controlling genotoxic impurities

- *PhRMA methodology:* It offers structural categorization in the form of functional group alerts. It was previously shown that the existence of these structural moieties contributed to DNA mutation.
  - Group 1: Aromatic groups: N-hydroxyaryls, N-acylated amino-aryls, aza-aryl N-oxides, amino-aryls, alkylated amino-aryls, purines, pyrimidines, intercalators, PNAs or PNAHs, etc.
  - Group 2: Alkyl and aryl groups include nitro compounds, carbamates epoxides, aldehydes, N-methylols, and N-nitrosamines.
  - Group 3: Hetero aromatic groups, such as primary halides, haloalkenes, and alkyl esters of phosphonates or sulfonates [44].

- *ICH guidance:* According to ICH (Q3B (R2)) criteria, impurity qualification threshold limits are determined as a percentage of the total daily consumption of the drug substance. They conceal imperfections in pharmaceutical compounds and products [45]. The guidelines also permit the use of different criteria for qualifying; lower limits may be suitable in cases where the impurity is classified as unusually toxic, a categorization that is pertinent to the case of genotoxic impurities. The risk assessment strategies are given in **Table 2**.

Table 2. Risk assessment and control testing for genotoxic contaminants

Key Topic	Guidelines
Regulations for genotoxic impurity control	Position paper from PhRMA: An explanation for identifying, evaluating, and managing certain contaminants in drugs that have the potential to cause genotoxicity.
	EMA: Protocol on the emissions of toxic or hazardous materials. The notion and values for the threshold of toxicological concern (TTC) were also introduced.
	FDA industry guidance: Suggested methods for handling genotoxic and carcinogenic impurities in drug substances and products generally following the EMA recommendation.
	Plan for ICH M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. This document is being developed and might eventually supersede the current FDA and EMA recommendations.
Test guidelines for genotoxicity	Pharmacies designed for human use: Genotoxicity testing and data interpretation (ICH S2. ICH S2A (1996) and S2B (2007), which serves as the worldwide guide for testing for genotoxicity.
	EMA: 2008 guideline for the evaluation of herbal substances and preparations genotoxicity. This guideline outlines a broad framework, useful methods, and interpretation guidelines for assessing the possible genotoxicity of herbal medicines and preparations.
Evaluation of genotoxic and carcinogenic chemical risks	General methodologies and approaches for genotoxic and carcinogenic substances: European Commission Health & Consumer Protection Directorate (2009).

- *EU guidance*: CHMP (2006) guideline offers suggestions on how to get approval for novel molecular entities and active compounds that are already approved but have undergone modifications that might introduce new or greater amounts of genotoxic contaminants, such as new synthetic pathways. The CHMP guideline's main focus is on substances that are reactive to DNA and have the potential to damage DNA directly. If a substance-specific calculation cannot be done, the guideline suggests utilizing a Threshold of Toxicological Concern. The TTC is based on a probability distribution of carcinogenic potencies to determine the daily exposure level (mg/person) of most carcinogens that would result in a cancer risk of less than 1 in a million throughout a person's lifetime i.e., 1.5 mg/d intake of a genotoxic impurity is considered to be associated with an acceptable risk for most pharmaceuticals, according to the CHMP recommendation.
- *USFDA guidance*: Regarding acceptable levels, and suggested methods for identifying and addressing these contaminants and their breadth, the USFDA and CHMP guidelines appear to be very comparable. The advice aims to deal with identified and expected API and synthetic process pollutants that arise during clinical development and in tasks related to new marketing applications. The threshold for acceptable impurities can be reduced to a level that aligns with a daily consumption of 1.5 mg/d at the Tolerable Daily Intake level. The USFDA stated that a staged-TTC approach is suitable for supporting shorter-term exposures in clinical development due to the variability in the length of clinical trials, the use of risk estimates from lifetime rodent assays, the application of conservative assumptions, and the limitations manufacturers face in detecting and controlling impurities during early development [46].

Methods for genotoxic impurity assessment and analysis

Because genotoxic contaminants must be analyzed at much lower values than 0.01–0.03%, this can be an extremely difficult task. For the analytical process, the ideal detection limits are between 1 and 5 ppm. Moreover, genotoxic pollutants react, which complicates sampling and calls for additional safety precautions. The most common methods used nowadays for analyzing genotoxic impurities are GC and HPLC [47]. The various analytical methods used for assessing are depicted in **Figure 4**.

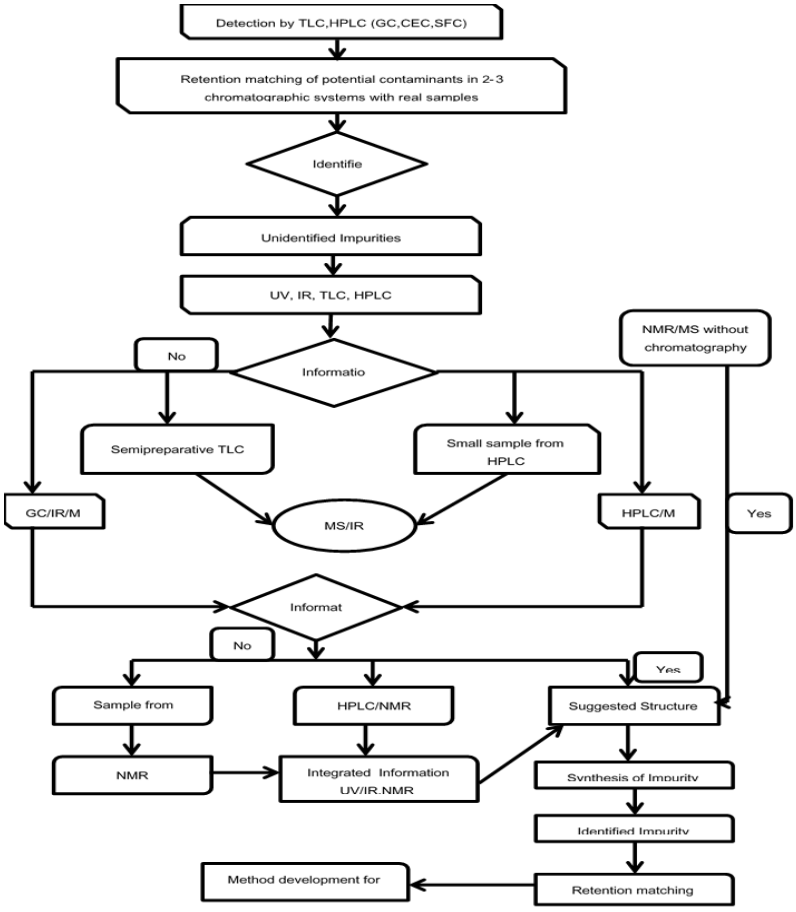


Figure 4. Analytical profiling for drug impurity

- *High-performance liquid chromatography*: HPLC's appeal for analyzing non-volatile genotoxic chemicals is mostly due to its user-friendly nature. Because the pollutants' and the API's structures are comparable, the HPLC technology's great selectivity and accurate measurement are demonstrated. These days, reversed-phase HPCL is also often used. HPLC's appeal for analyzing non-volatile genotoxic chemicals is mostly due to its user-friendly nature. To enhance the sensitivity and selectivity of trace amounts of GTIs, other detectors can be used Dalfampridine, five potential genotoxic impurities were found by employing the HILIC Technique in conjunction with HPLC. For impurities I, II, III, IV, and V its sensitivity allows it to detect them at low concentrations of up to 7.5 ppm as shown in **Figure 5a**.
  Chloramphenicol, application of 2, 4-DNPH derivatization was used to determine 4-nitrobenzaldehyde in injectable formulations using the HPLC-UV derivatization analytical technique and the drug's 4-nitrophenyl hydrazone was converted to 3-nitrophenyl hydrazone as shown in the **Figure 5b** [48].
- *Gas chromatography:* GC-MS and static headspace gas chromatography are considered the most effective methods for analyzing various genotoxic contaminants like halides, sulfonates, and epoxides. The gas chromatography headspace technique is commonly utilized for residual solvent examination in quality control labs globally because of its tight adherence to ICH Q3C standards. Tablets containing valsartan include the impurity N-nitrosodimethylamine. This impurity is considered a human carcinogen due to its presence in the drug substance's production process and its subsequent presence in the final product. The drug substance and drug product Valsartan was found to contain NDMA using the GC/MS headspace method. Valsartan tablets are taken off the market based on the LOQ and LOD criteria of 0.3 and 0.05 respectively as shown in **Figure 5c**.
- *Liquid chromatography and mass spectroscopy:* LC-MS is a flexible tool used to elucidate the structure of contaminants. Fragmentation of the drug's mass aids in identifying and analyzing unfamiliar impurities. It provides fast and effective separation. The source of the rise in impurities is revealed by the LC-MS structural elucidation data, which will help to lower the level of contaminants in the pharmaceutical product. The application of LC-MS/MS in the research led to increased efficiency, decreased cost per analysis, an extremely low quantitation limit, and the identification of genotoxic substances in minute amounts. Consequently, the 2-butyl p-toluenesulfonate of the medication had to be identified, a process that took a long time and involved HPLC and GC analysis. Trace levels specifically 1 ppm of a contaminant in naproxen, were quantitatively evaluated utilizing the triple quadrupole LCMS technique as shown in **Figure 5d** [49].
- *Inductively coupled plasma mass spectrometry (ICP-MS) with optical emission spectroscopy (ICP-OES)*: ICP-MS with ICP-OES is a robust multi-element technique for examining metal pollutants that can change DNA. In the determination of elemental impurities with either method, a sample can be evaluated in three ways directly, after sample preparation by dissolving it in an aqueous or organic solvent [50, 51].
- *Nuclear magnetic resonance spectroscopy*: NMR is valuable for its ability to offer distinctive insights into the stereochemistry and bonding inside molecules. To characterize genotoxic impurities and degradants at very low concentrations, structural analysis is required, because NMR is non-invasive and non-destructive it can be used to characterize pollutants and degradants existing at very low levels.

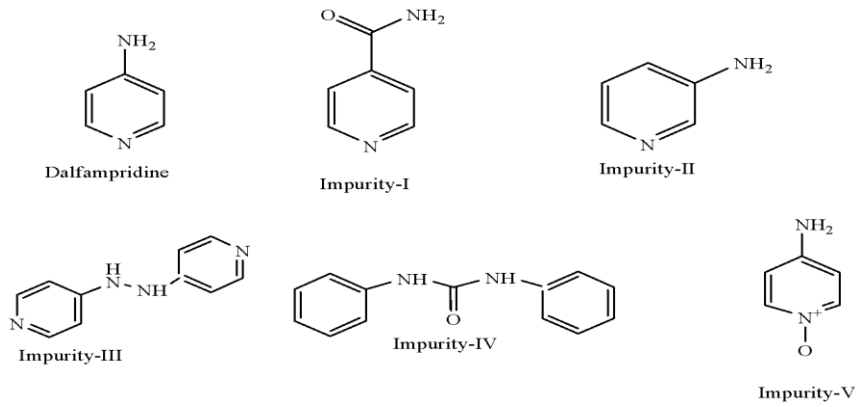
Pharmaceutical product recalls related to carcinogenic and genotoxic substances.

In recent years, several pharmaceutical companies have recalled popular medications for diabetes, heartburn, and hypertension due to the presence of nitrosamines, which are carcinogenic and genotoxic substances that have been connected to cancer in animal studies. In the last two years, more than 1400 product lots have been recalled or taken off the market because their nitrosamine content exceeded the daily allowed limit. When nitrosamine impurities were found to be present more than the threshold level in several pharmaceutical goods utilizing APIs, including valsartan, irbesartan, losartan, metformin, ranitidine, and nizatidine, the items were recalled or taken out of distribution [52].

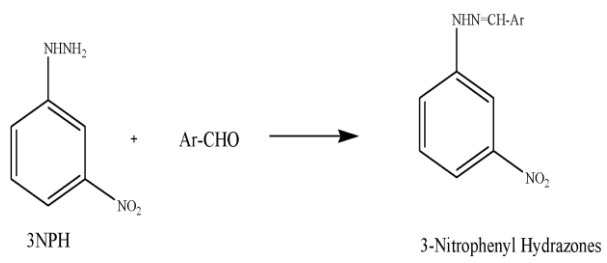
- *Valsartan:* The 2018 nitrosamine recalls initially affected Valsartan, a well-known angiotensin II receptor blocker. Novartis was the company behind the development of Diovan, a valsartan-based high-blood pressure treatment. Generic versions of ARB pharmaceuticals of nitrosamine impurities, such as NDMA and NDEA [53].
- *Irbesartan*: Lupin Pharmaceuticals withdrew several batches of hypertension medications irbesartan and irbesartan/hydrochlorothiazide in October 2021 because they contained high quantities of N-nitroso

irbesartan. Sanofi is the company that develops and markets irbesartan under the Avapro brand. The first generic versions of irbesartan became available approximately ten years ago.

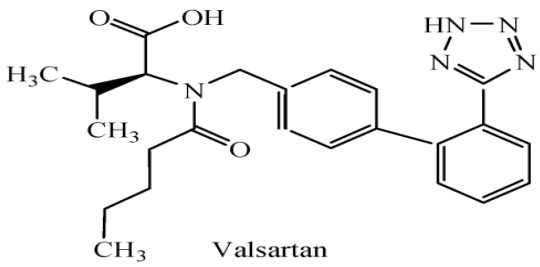
- *Nizatidine*: Nizatidine is used to treat gastric reflux disease, duodenal ulcers, and dangerous ulcerative esophagitis. Mylan issued a countrywide recall of three lots of nizatidine from Solara Active Pharma Sciences in January 2020 due to the presence of NDMA in the active component. In April, Amneal Pharmaceuticals voluntarily recalled significant quantities of nizatidine oral solution.
- *Quinapril*: Lupin Pharmaceuticals withdrew four packets of the blood pressure medicine quinapril in December 2022 owing to nitrosamine-related concerns. Pfizer recalled five batches of quinapril pills in March 2022 because they had high quantities of the nitrosamine N-nitroso-quinapril. Pfizer has also recalled a significant amount of quinapril/hydrochlorothiazide sold under the Accuretic brand.
- *Rifampin and rifapentine*: Rifampin is used in conjunction with other drugs to treat tuberculosis in different parts of the body. In 2020, the FDA revealed that multiple batches of the medications rifampin and rifapentine included nitrosamine impurities. Thus, to mitigate shortages, the EPA authorized many medications containing elevated levels of 1-methyl-4-nitrosopiperazine or 1-cyclopentyl-4-nitrosopiperazine as shown in **Figure 5e**.
- *Sitagliptin*: Merck & Co. revealed in August that nitrosamine was found in samples of their sitagliptin-containing medications, Januvia, Janumet, and Steglujan. If sitagliptin's nitroso-STG-19 levels exceed the advised threshold, the FDA announced that it might be temporarily dispensed.
- *Varenicline*: Pfizer ceased exporting Chantix to foreign nations in June 2021 due to the discovery of the nitrosamine N-nitroso-varenicline. A few months later, the company decided to expand the recall. In February 2022, a federal judge dismissed a proposed lawsuit because N-nitroso-varenicline was found in some batches of the smoking cessation product as shown in **Figure 5f**.
- *Losartan*: Losartan is categorized as an angiotensin II receptor blocker. Nitrosamine contamination led to Torrent Pharmaceuticals recalling a large number of losartan potassium and losartan potassium/hydrochlorothiazide pills in 2019. Hetero Labs Ltd.'s active pharmaceutical ingredient was accused of being connected to the problem by Torrent Pharmaceuticals. The year 1995 saw Merck get approval to market losartan potassium, an angiotensin II inhibitor, under the brand name Cozaar. As shown in **Figure 5g**.
- *Metformin*: Metformin is used to treat high blood sugar, sometimes in combination with other medications, a nutritious diet, and frequent exercise. Individuals with diabetes type 2 utilize it. In 2020, the FDA found NDMA in the diabetic medication metformin.



a)



b)



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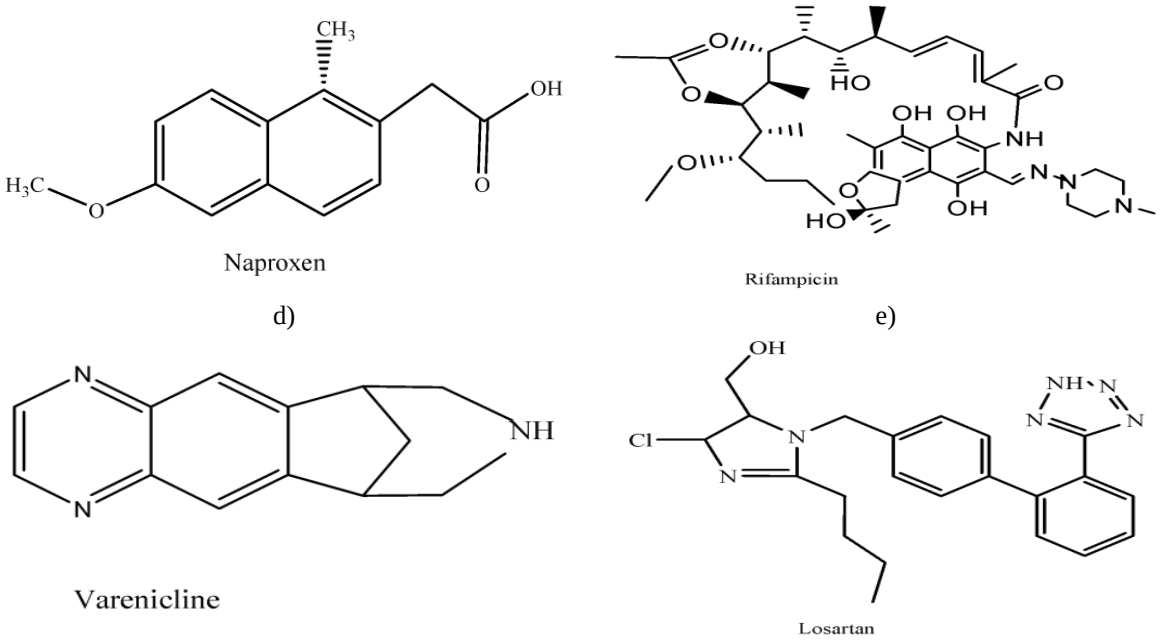

Figure 5. Structure of a) Dalfampridine and its impurities, b) 3-Nitrophenylhydrazone, c) Valsartan, d) naproxen, e) Rifampicin, f) Varenicline, and g) Losartan

- *Ranitidine*: This medicine decreases the amount of acid the stomach produces. The over-the-counter medication ranitidine, which is used to treat heartburn, was found to contain higher concentrations of nitrosamines, such as NDMA, in certain batches in 2019. The FDA eventually requested that the producers of ranitidine take the drug off of distribution when the lab Valisure found NDMA in it.

CONCLUSION

This review paper provides an overview of contaminants found in drug substances and drug products. It offers helpful details on the various kinds of impurities, their classification, origins, and techniques for isolating and describing them in addition to recognizing and classifying genotoxic impurities. According to the findings of this study, new drugs, pharmaceuticals, and individuals will have access to a safety regulatory framework through the identification and measurement of GTIs utilizing accurate, sensitive, and quantitative approaches. A few control techniques were also assembled to aid in the management of GTIs throughout the preliminary stages of drug development. Reading the complete discussion about impurity profiling and many related subjects that were presented above would be of a general and larger interest.

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