

A COMPREHENSIVE REVIEW OF NITROSAMINE CONTAMINATION IN PHARMACEUTICALS

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Abstract :

Nitrosamines are a group of chemicals that can cause cancer and mutations. They have impurities that can lead to cancer in parts of the body. In medicine nitrosamines usually form when certain amines mix with chemicals like nitrous acid. This acid can be made from nitrites in environments. Contamination can happen during the making of medicines. In 2018 some medicines called sartans were found to have levels of bad chemicals like NDMA and NDEA. This was a problem that led to many recalls and stricter rules. Later more medicines like metformin, ranitidine, rifampicin, duloxetine and varenicline were also found to have these chemicals. As a result groups like the FDA, EMA and WHO made new rules to control the problem. These rules include limits on how much of these chemicals can be in medicines between 18 and 96 ng/day. There are also steps that companies must follow to reduce the risk. These steps include checking for the chemicals testing to confirm and making changes to how medicines made. New technologies, like high-resolution mass spectrometry, GC-MS and LC-MS/MS can now detect these chemicals in small amounts. Companies are also finding ways to prevent the chemicals from forming. Some of these ways include changing how medicines are made using helpers that soak up nitrites and using ingredients that have low levels of nitrites. These methods are always being improved.

Key words : Nitrosamine, Nitrosating agents, NDMA , NDEA , Analytical methods, Impurities

INTRODUCTION

Nitrosamines are a group of chemicals that can cause problems. They can change the way our cells work and even cause cancer. The N-nitrosamines are especially bad because they can cause cancer in parts of our body like the liver, lungs, esophagus, pancreas and breast. We can find amounts of nitrosamines in some foods, drinks and in the air we breathe.. We do not want them in our medicine because they can hurt us if we take them for a long time. Nitrosamines can form in medicine when some chemicals mix together. This happens when amines and nitrosating agents like acid react. Nitrous acid usually comes from nitrites when they are in an environment. These bad chemicals can get into our medicine at stages of how it is made. They can form when the main ingredients are being made or they can show up when the ingredients start to break down. Sometimes they can even get into the medicine from the solvents, reagents and catalysts that are used to make it. For example if the same solvents and catalysts are used over and over or if the conditions are not just right like the temperature or the order in which the ingredients are added then nitrosamines can form. Even the way the medicine is packaged can sometimes cause problems, with nitrosamines. (Berardi et al., 2023)

A big problem happened in 2018. This is when people found out that some medicines had things in them called N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). These medicines were called medications. When people found out they had to recall these medicines from over the world. The people in charge of making sure medicines are safe got very serious about checking these medicines. metformin, ranitidine, rifampicin, duloxetine and varenicline. This is a deal because it can hurt peoples health. The people

in charge like the U.S. FDA, EMA and WHO had to make new rules to keep people safe. The FDA and other people in charge said that we need to do three things to make medicines safe. First we need to check if the medicines have these things in them. Second we need to test them to make sure. Third we need to change how we make these medicines so they do not have these things. We want to make sure that medicines do not have N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in them before people take them. The people in charge also said how much of these things is okay to have in medicines. This is usually between 26.5 and 96 ng/day. We use tools to check for N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in medicines. These tools are called LC-MS/MS, GC-MS and HRMS. They help us make sure that medicines are safe for people to take. We also do things to keep medicines safe. We add things to medicines to stop N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) from forming. We change the way we make medicines and how we store them.

N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) are bad for people. They can cause cancer. That is why we need to be very careful. We have found N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in medicines like valsartan, ranitidine and metformin. This is why some medicines were recalled. N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) can form when we make medicines or when we store them. They can even form when some things in medicines react with each other. This makes it hard to find them. Stop them. To solve this problem we need to do things. The people who make medicines need to check everything they do to make sure N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) do not form. They need to avoid using things that can make N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). They need to make medicines in a way that stops N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) from forming. They need to use tools to check for N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). The people in charge, like the FDA, EMA and WHO said how much of these things is okay to have in medicines. They also said when the people who make medicines need to check for N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). (FDA, 2023; WHO, 2019).

The current need for continued research into nitrosamine impurities is greater than ever. As newer drug products and formulations enter the market, there's a growing concern about the formation of novel nitrosamine drug substance-related impurities (NDSRIs), especially during long-term storage under stress conditions (ICH M7(R2), 2024). The complexity of these impurities and the evolving nature of excipient interactions require deeper mechanistic understanding and predictive modeling to prevent contamination before it occurs. In addition, the pharmaceutical industry must continue to invest in innovative detection technologies and green chemistry solutions that can effectively mitigate nitrosamine risks without compromising drug efficacy or stability. Therefore, addressing nitrosamine impurities is not just a regulatory obligation but a critical component of ensuring drug safety, regulatory compliance, and patient trust.

The purpose of this review is to provide a comprehensive overview of nitrosamine impurities in pharmaceuticals, covering their sources, mechanisms of formation, analytical detection, regulatory frameworks, toxicological profiles, and strategies for risk mitigation. By synthesizing recent developments and case studies, this paper aims to support industry stakeholders and regulatory authorities in minimizing nitrosamine-related risks, thereby ensuring the continued safety, efficacy, and quality of medicinal products. (Pal & B., 2024).

Different types of Nitrosamine Impurities : (Dhansekar et al., 2025)

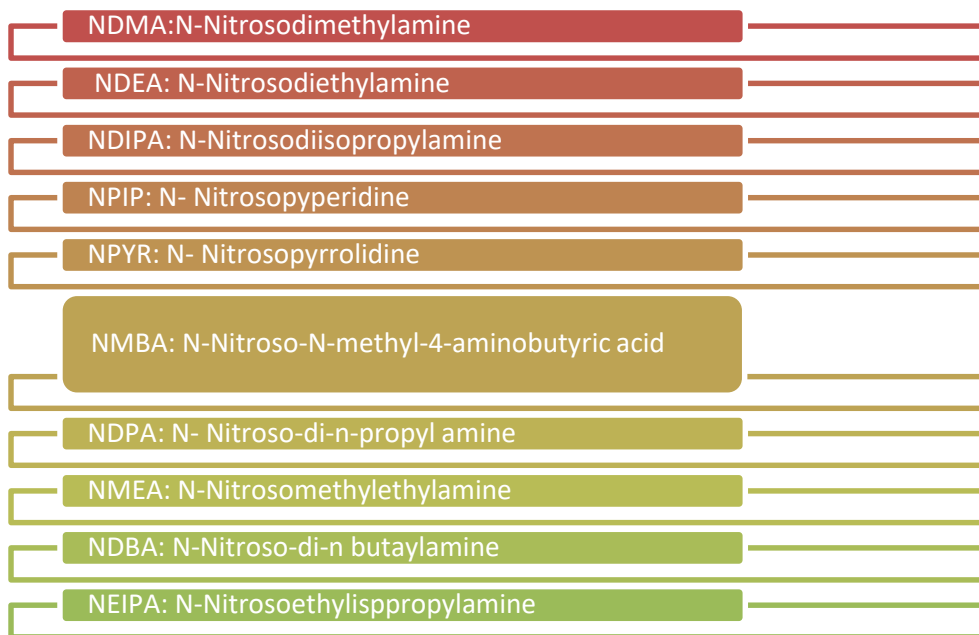


Figure 1 : Types of Impurities

Risk Factors Associated With N-Nitrosamine Formation (Cioc Et Al., 2023)

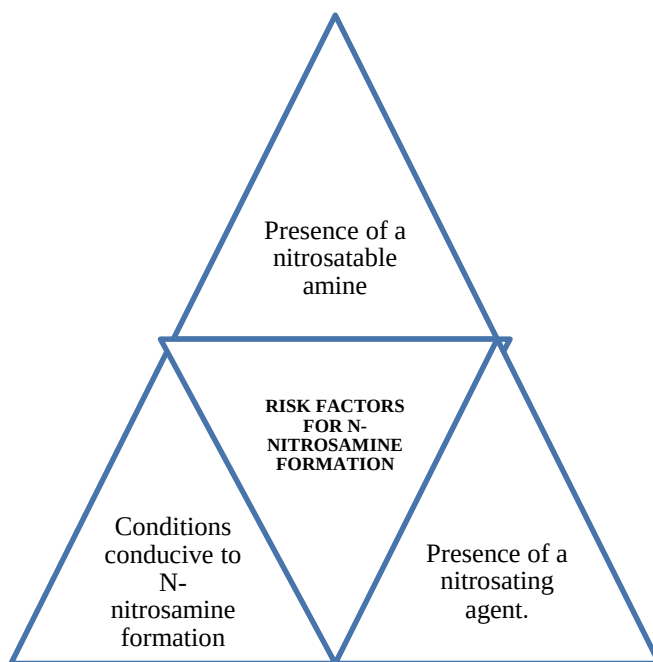


Figure 2 : Risk factor for Nitrosamine Impurities

Sources of Nitrosamines Formation (Akkaraju et al., 2023)

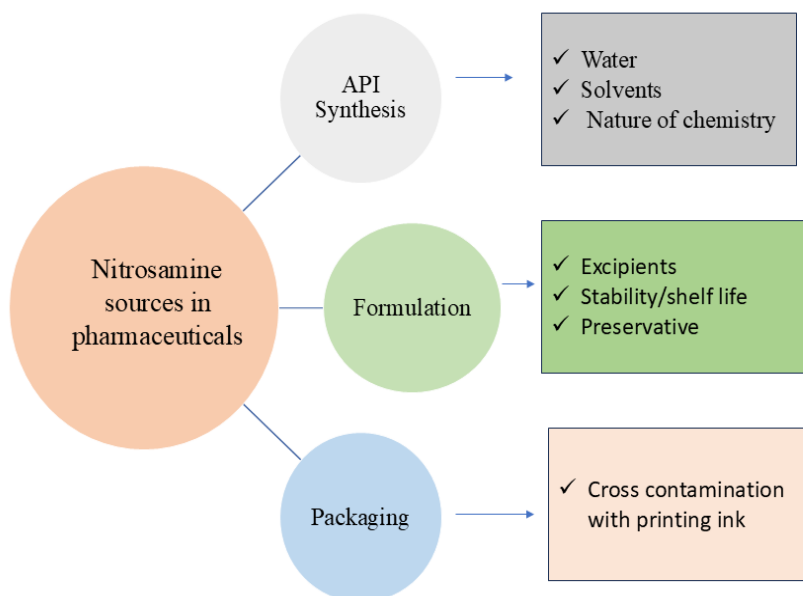


Figure 3: Sources of Nitrosamine

Mechanism of Nitrosamine formation

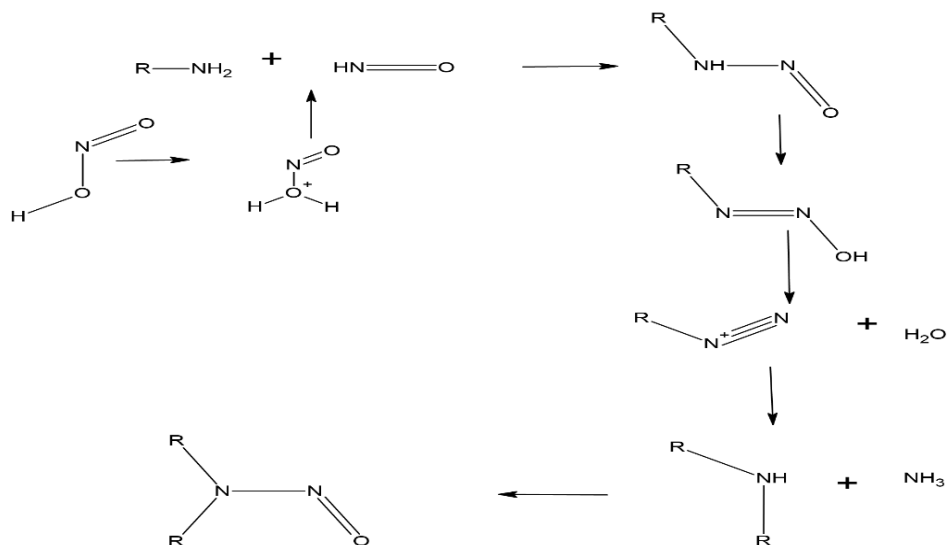
They result from the reaction of nitrite in acidic environments with a secondary or tertiary amine. Nitrous acid is inherently unstable but can be generated in situ from nitrites (NO_2) in acidic environments. There are multiple possible sources of nitrosating agents ($+\text{NO}$) and reactive amines in pharmaceutical formulations. Nitrosating agents can include nitrite salts (such as NaNO_2), nitrous acid (HNO_2), nitrous acid anhydride (N_2O_3), or nitrogen oxides (for example, NO_2). (Khan et al., 2023) When drug products contain carbamates, amide, N-alkyl amide, secondary or tertiary, and quaternary ammonium salts, nitrosamine impurities are created. The kind, composition, and concentration of the nitrosating agent determine how much nitrosamine is present. Compared to other nitrosating agents, secondary amide is more reactive.

Recovered solvent and catalyst utilized in the process result in the creation of Nitrosamine impurities. These solvents, when treated with nitrites or nitric acid, aim to eliminate residual azide, resulting in the creation of Nitrosamine impurities. Another possible source of nitrosamine impurities is the lack of optimization in the API manufacturing process, particularly when reaction conditions, like temperature, pH, or the order of adding reagents, intermediates, or solvents, are unsuitable or inadequately controlled.

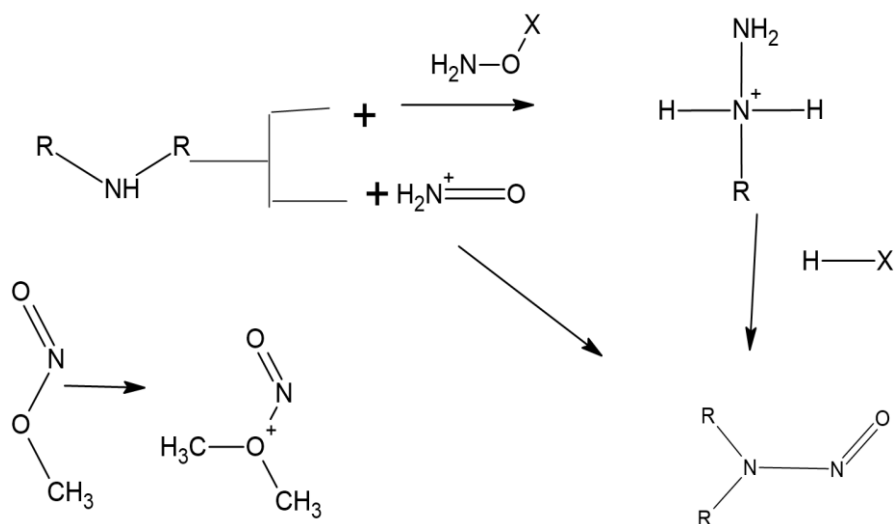
Nitrosation Pathways of Nitrosamine Formation

Nitrosamines are typically produced through the nitrosation of an amine precursor that is present alongside a nitrosating agent. In extremely acidic environments that reduce amine reactivity, these contaminants can be reduced. While the nitrosation process is promoted at acidic pH, it becomes less reactive at extremely low pH levels because of the protonation of amines. The ideal state for nitrosation aligns the pH with the basicity of the amine precursors. The majority of nitrosating agents employed in API synthesis are the oxidized.

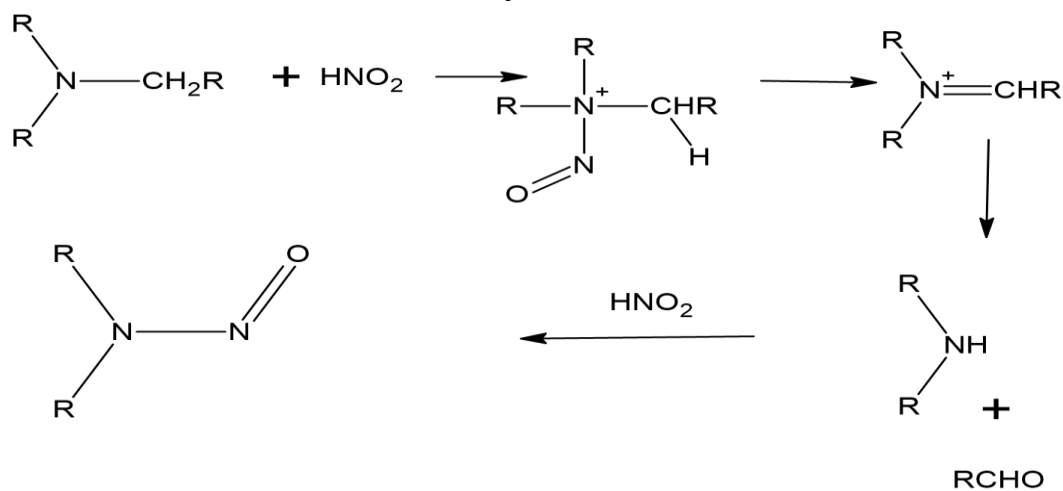
The potential mechanisms for the nitrosation of primary, secondary, and tertiary amines as well as ammonium salts are



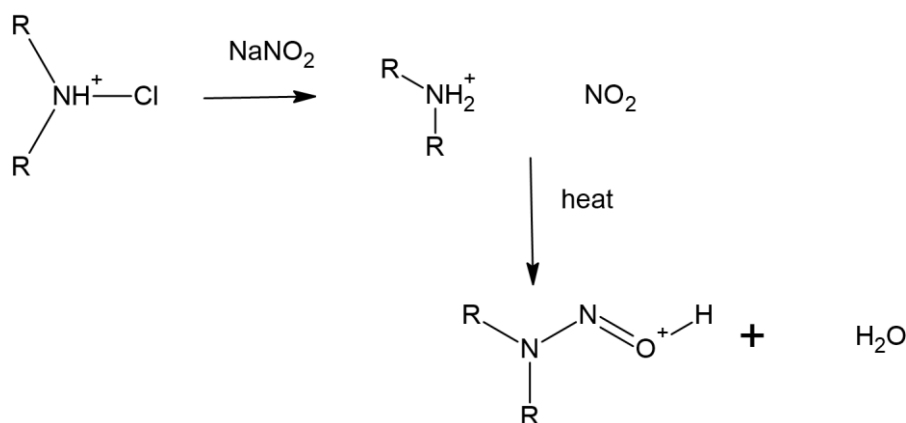
Primary Amine



Secondary Amines



Tertiary Amines



Quaternary Amines

Pathways of nitrosamine formation between nitrosating agents and primary, secondary, and tertiary amines and ammonium salts.

Table 1: Amine And Amide Precursors of Nitrosamine Impurities in Pharmaceuticals

Nitrosamines	Amine and Amide Precursors
N-nitrosodimethylamine (NDMA)	N,N-dimethyl formamide (DMF) dimethylamine (DMA) trimethylamine (TMA)
N-nitrosodiethylamine (NDEA)	N,N-diethylamine (DEA) triethylamine (TEA)
N-nitroso-N-methyl-4-aminobutyric acid (NMBA)	N-methylamino-N-butyric acid (MBA)
N-nitrosodiisopropylamine (NDIPA)	N,N-diisopropylethylamine (DIPEA)
N-nitrosoethylisopropylamine (NEIPA)	N,N-diisopropylethylamine (DIPEA)
N-nitrosodibutylamine (NDBA)	N,N-dibutylamine (DBA)
1-methyl-4-nitrosopiperazine (MNP)	1-methyl piperazine
1-cyclopentyl-4-nitrosopiperazine (CPNP)	1-cyclopentyl piperazine

History of Contamination issue

The drug from which nitrosamine contaminants are derived:

- Sartan medicine
- Rifampicin drug
- Ranitidine
- Metformin - Containing medicine
- Champix medicine

Table 2 : Impurities Detected in Drugs

Drug from which nitrosamine impurities derived	Type of Impurity	Reported year	Reason for formation
Sartan (Losartan, Valsartan) (Glowienke et al., 2022)	N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA)	2018	manufacturing process, often due to specific chemical reactions involving solvents, reagents, and certain conditions used in the synthesis of the sartan molecule, especially when a "tetrazole" ring structure is present;
Rifampicin	1-methyl-4-nitrosopiperazine (MNP)	2020	presence of a secondary amine group within its chemical structure, which can react with nitrite ions (potentially present in solvents, reagents, or even during manufacturing processes) under certain conditions like elevated temperature or pH
Ranitidine	N-nitrosodimethylamine (NDMA),	2019	slow degradation process within the molecule itself
Metformin	N-nitrosodimethylamine (NDMA), other nitrosamine impurities that may be present in smaller amounts include N-nitrosodiethylamine (NDEA), N-nitroso-diisopropylamine (NDIPA), and N-nitrosomethylphenylamine (NMPA)	Late 2019	due to a slow degradation process within the molecule itself
Champix	N-nitroso-varenicline.	2021	potential issues within the

			manufacturing process
Duloxetine	N-nitroso-duloxetine.	2024	Manufacturing process of drug, chemical structure, storage condition

Key Guidance Documents and Communications on Nitrosamines

Regulatory Authorities of the NISG have released various essential guidance documents and communications to disseminate pertinent information regarding nitrosamines. Additionally, regulatory authorities have implemented market actions like recalls since 2018 as nitrosamine impurities were detected and measured at levels surpassing the acceptable intake (AI) limit in various drug products. Moreover, concerning drug products have been recognized on agency websites and in peer-reviewed scientific journal publications. The verified existence of nitrosamines in angiotensin-II receptor antagonists, referred to as “sartans,” prompted regulatory authorities to take initial actions regarding this issue. Once the “sartan” TagedEndTagedPcases were validated, numerous regulatory bodies implemented measures like recalls for metformin and ranitidine medications. Primarily drawing from the insights obtained with “sartans,” numerous regulatory bodies outlined standard factors to assess quality risks and manage the risk of nitrosamines in APIs and pharmaceutical products for human consumption, initiating in 2019 within certain regions. A widespread three-step method has been adopted by the majority of regulatory agencies to direct the industry on evaluating and controlling nitrosamines in sold drug products. (Horne et al., 2023)

Regulatory landscape

Since mid-2018, regulators worldwide including the FDA and EMA have taken concerted action after nitrosamines, such as NDMA and NDEA (probable animal carcinogens), were unexpectedly found in drug products like sartans, ranitidine, metformin, and duloxetine. In the EU, EMA’s CHMP invoked Article 5(3) of Regulation (EC) 726/2004 and launched a comprehensive scientific review in June 2020, followed by binding guidance in July to MAHs (Marketing Authorisation Holders) requiring risk evaluation, confirmatory testing, and process updates to eliminate or minimize nitrosamine levels.

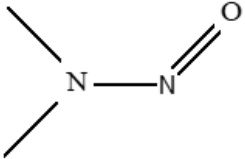
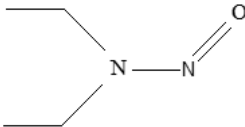
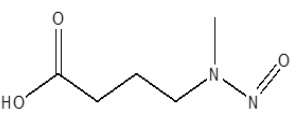
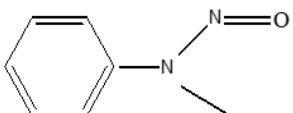
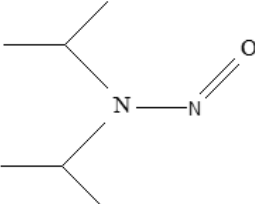
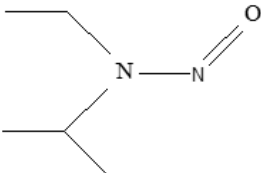
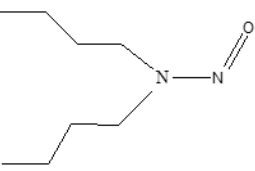
In the U.S., FDA released its final “Control of Nitrosamine Impurities in Human Drugs” guidance in February 2021 (updated regularly), recommending that manufacturers conduct risk assessments, root-cause analyses, sensitive testing, and implement appropriate mitigation strategies including reformulation and analytical methods depending on impurity type (i.e., small-molecule vs. drug-substance-related nitrosamines) FDA also introduced the Carcinogenic Potency Categorization Approach (CPCA) to set Acceptable Intake (AI) limits when data are insufficient, and defined timelines such as risk assessment deadlines by March 2021 and submission of changes by October 2023–August 2025, depending on impurity class.

International harmonization is ongoing: the ICH M7(R1)/(R2) guidelines classify nitrosamines as “cohort of concern” mutagenic impurities and require use of the TTC concept (~1.5 µg/day) when specific data are lacking; ICH is now working on a global addendum to unify nitrosamine control approaches.

In addition to regulatory updates, health agencies have overseen numerous strategic recalls (e.g., sartans, ranitidine, metformin, duloxetine), issued surveillance and vigilance alerts, and collaborated internationally via groups including Health Canada, TGA, and PMDA to monitor emerging cases and refine pharmacopoeial methods.

Acceptable Intake limits recommended by FDA as given (Kruhlak et al., 2024)

Table 3 : Intake limit of Nitrosamine Impurities

Nitrosamine	Structure	Acceptable intake Limit (ng/day)
NDMA (N-Nitrosodimethylamine)		96
8NDEA (N-Nitrosodiethylamine)		26.5
NMBA (Nitrosomethyl-N-butylamine)		96
NMPA (N-Nitrosomethylphenylamine)		34.3
NIPEA (N-Nitrosoisopropylethylamine)		26.5
NDIPA (N-Nitrosodiisopropylamine)		26.5
NDBA (N-Nitrosodibutylamine)		26.5

(<https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs>, n.d.)

Recall of Pharmaceutical product :

There has been a rise in recall of pharmaceutical product due to nitrosamine contaminants. This has become a widespread issue, and producers must prepare for any emergencies that could result from ineffective product recalls. Regulatory agencies and patients alike are highly troubled by the presence of nitrosamine contaminants in prescription products. More than 1400 product batches have been recalled or withdrawn, due to the presence of nitrosamines exceeding the daily allowable limit, from the markets over the last two years.

Many pharmaceutical items containing the APIs valsartan, irbesartan, losartan, metformin, ranitidine, and nizatidine were taken off the market or recalled due to findings that these medications contained three distinct types of nitrosamine impurities. To prevent a shortage in the market, the FDA chose not to withdraw the life-saving medications rifampin and rifapentine. The sources of MNP and CPNP for these medicinal products are still under investigation. As per the FDA, the acceptable daily nitrosamine intake for the majority of individuals is between 26.5 and 96 ng each day. Losartan is associated with the highest number of product recalls (nearly 24% of the overall total), as 324 batches of this medication have been removed from the market. An thorough examination of the FDA data reveals that drug products with "sartans" account for nearly 81% of the recalls. Besides sartans, combo products with sartans blended with other APIs made up about 42% of the overall total. Many blood pressure medications are being recalled by Pfizer and other companies due to higher levels of nitrosamine. (Bharate, 2021; Nori et al., 2023)

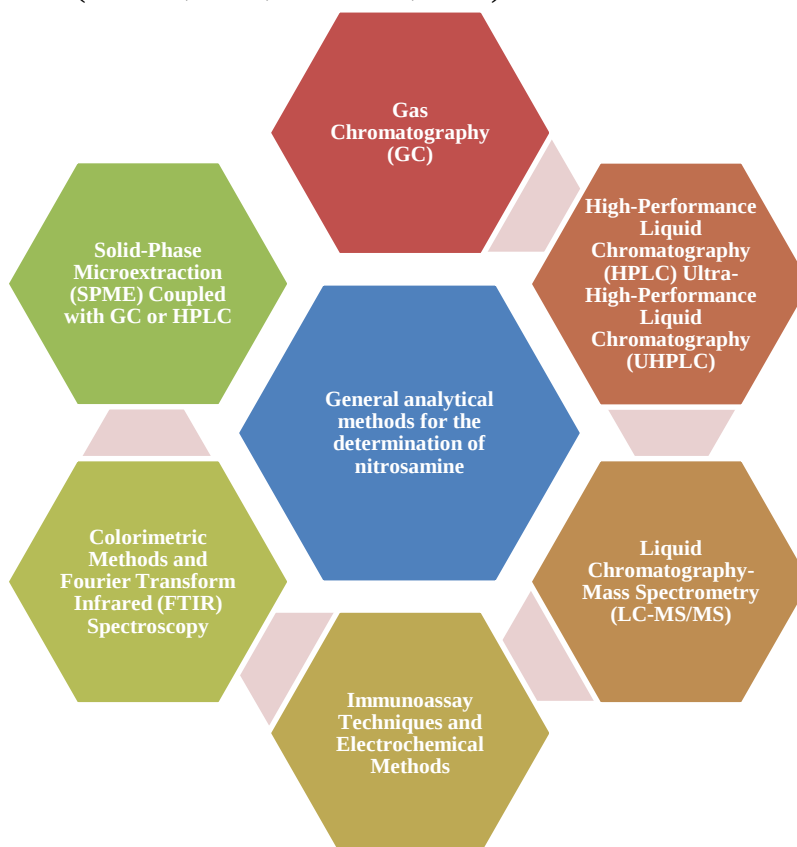


Figure 4: Analytical Methods For the Determination of Nitrosamine

Liquid Chromatography-Mass Spectrometry (LC-MS) is a highly effective analytical method for the accurate identification and quantification of nitrosamines, especially in complex matrices. Its high sensitivity and selectivity make it a preferred choice among industries and regulatory bodies concerned with nitrosamine contamination. Gas Chromatography-Mass Spectrometry (GC-MS) also serves as a reliable method, particularly suitable when nitrosamine compounds are volatile. LC-MS exhibits exceptional separation and detection capabilities for a wide range of nitrosamines (Daripelli et al., 2024; Pathak et al., 2025; Thumbar et al., 2025).

High-Performance Liquid Chromatography (HPLC) is widely employed in nitrosamine analysis due to its adaptability to various sample matrices. Innovations in instrumentation and column chemistry have led to reduced analysis time and enhanced sensitivity. Tandem Mass Spectrometry (MS/MS), including techniques like triple quadrupole and ion trap mass spectrometers, offers enhanced selectivity and reduced interference, making it particularly valuable for trace-level detection of nitrosamines. Furthermore, High-Resolution Mass Spectrometry (HRMS) instruments such as Orbitrap and time-of-flight (TOF) mass spectrometers provide precise mass measurements, which are essential for identifying nitrosamine contaminants in complex samples. The use of HRMS in nitrosamine analysis is increasingly gaining traction.

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-destructive analytical technique that provides detailed molecular structural information of nitrosamines, offering valuable insights into their chemical composition. Additionally, immunological assays such as enzyme-linked immunosorbent assays (ELISA) are employed for high-throughput nitrosamine screening. Although not as precise as mass spectrometry, these assays are useful as preliminary screening tools (Detecting Nitrosamine Impurities: Advances in Analytical Techniques, n.d.).

Gas Chromatography (GC) is another established method for nitrosamine detection. GC-MS, which combines the separating capabilities of gas chromatography with the detection power of mass spectrometry, is commonly used for identifying and quantifying nitrosamines based on their mass-to-charge ratio. It offers high sensitivity and selectivity, making it capable of detecting nitrosamines at trace levels. Sample preparation often involves techniques like solid-phase extraction (SPE) or liquid-liquid extraction (LLE). Additionally, GC coupled with a Nitrogen-Phosphorus Detector (GC-NPD) is effective for detecting nitrogen-containing compounds such as nitrosamines, offering sensitivity and operational simplicity (Selaya et al., 2025).

Risk Assessment and Toxicity of Nitrosamine

Possible health hazard due to Nitrosamine impurities

Risk Associated with cancer:

N-nitrosamines are not limited to pharmaceuticals and can also be present in drinking water, processed foods, cured meats, fish, and tobacco smoke. The average daily exposure to N-nitrosamines from dietary and environmental sources is estimated to be around 1.9 – 25.0 µg/day. It is believed that this exposure may account for approximately 6,000 cancer cases per 1 million individuals. Specifically, the N-nitrosamine impurities in valsartan have been linked to a slight increase in the incidence of hepatic, colorectal, and uterine cancers. Although the number of rifampin prescriptions is significantly lower, with an estimated 6,000 patients in the US receiving rifampin as part of a 4- or 6-month TB treatment regimen in 2020, the potential risk of exposure to N-nitrosamine impurities following rifampin use remains substantial. (Paglialunga & Van Haarst, 2023)

Lung cancer

Nitrosamine impurities can trigger lung cancer by promoting cell proliferation, migration, and survival through several signaling pathways. They affect G-protein, nicotinic acetylcholine receptor (nAChR), and epidermal growth factor receptor (EGFR) signaling. Specifically, nitrosamines alter the EGFR-related cAMP-PKA-RAF-MEK-ERK and PI3K-Akt-mTOR pathways, boosting cancer cell growth and survival. They also influence nAChR signaling, calcium channels, and protein kinase C, aiding cancer cell migration. Additionally, nitrosamines activate beta-adrenergic signaling, promoting angiogenesis to support tumor growth. (Vikram et al., 2024)

Esophageal cancer

Esophageal cancer has a high incidence rate and is influenced by environmental factors, with nitrosamines playing a significant role in its development. Research by Zhao et al. found that patients with esophageal squamous cell carcinoma (ESCC), basal cell hyperplasia, and precancerous lesions had higher urinary levels of nitrosamines (NMEA, NDBA, NPYR, and NMOR) compared to healthy individuals. The study indicates a dose-response relationship between nitrosamine exposure and the increased risk of ESCC, highlighting the harmful effects of specific nitrosamines like NMEA, NDBA, and NPYR. (Zhao et al., 2020)

Hepatocellular and pancreatic cancer

Hepatocellular carcinoma, a deadly cancer, can be triggered by the metabolism of nitrosamines with the help of P450 enzymes. Lifestyle factors, like consuming foods with high levels of nitrosamines or N-nitroso compounds, contribute to cancer risk. These compounds create DNA damage in cells, particularly in the liver and digestive organs, leading to cancer. Processed foods with nitrosamine additives are a significant exposure source. A study by Zheng et al. found that higher intake of N-nitroso compounds increases the risk of

hepatocellular carcinoma, especially when combined with factors like hepatitis B or C infections or alcohol consumption.(Aschebrook-Kilfoy et al., 2011; Shephard et al., 1987)

Breast cancer

Breast cancer, a leading cause of death, is often linked to unhealthy lifestyle choices and diet. Tobacco contains nitrosamines like NNK, a potent carcinogen that promotes cancer development, including in breast cancer. NNK interacts with the α 9nAChR receptor in cancer cells, influencing cell proliferation through key signalling pathways such as MAPK and PI3K-Akt, often activated by nicotine and estrogen. This creates a feedback loop that contributes to cancer progression. Studies using gene knockdown techniques have confirmed the role of α 9nAChR in this process. However, the impact of nitrosamine impurities in pharmaceuticals on α 9nAChR activity and breast cancer development remains unexplored.(Ardenne & Reitnauer, 1975; Baker et al., 1975; Lee et al., 2010)

Table 4: The Five Predicted Carcinogenic Potency Categories and Associated Recommended AI Limits for NDSRIs

Potency Category	Recommended AI (ng/day)	Comments
1	26.5	The recommended AI limit of 26.5 ng/day matches the class-specific limit for nitrosamine impurities, derived from the most potent tested compound, NDEA. Category 1 NDSRIs are expected to have carcinogenic potency at or below this limit.
2	100	The recommended AI limit of 100 ng/day is based on two well-studied potent nitrosamines, NDMA (96 ng/day) and NNK (100 ng/day). Category 2 NDSRIs are expected to have carcinogenic potency no greater than these compounds.
3	400	Compared to Category 2, these NDSRIs have lower carcinogenic potency, often due to weakly deactivating structural features. Their recommended AI limit reflects a fourfold reduction in potency.
4	1500	Category 4 NDSRIs may undergo α -hydroxylation but are expected to have low carcinogenic potency due to unfavorable activation or efficient clearance. Their AI limit of 1500 ng/day is based on the TTC per ICH M7(R2).
5	1500	Category 5 NDSRIs are not expected to undergo α -hydroxylation due to steric hindrance, lack of α -hydrogens, or formation of unstable, non-DNA-reactive species. Their AI limit of 1500 ng/day follows the TTC per ICH M7(R2).

(ICH M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk - Scientific Guidelin, n.d.; The Guidance for Industry Control of Nitrosamine Impurities in Human Drugs, n.d.)

Molecular toxicity of nitrosamine impurities

Genotoxicity

NDMA, NDEA, N-nitrosoethylisopropylamine (NEIPA), NDIPA, N-nitrosodibutylamine (NDBA), and NMBA are the primary nitrosamines that can promote carcinogenic effects through genotoxicity or

mutagenicity within the biological system. Recently, it has become clear that contaminants in various pharmaceutical products present considerable risks to human health, as demonstrated in rodent studies. For instance, NMBA and NDEA exhibit metabolic similarities to NMDA and 12 carcinogenic N-nitrosomethyl-N-alkylamines (NMAs). Both genotoxicity and tumorigenicity are evaluated by analyzing chromosomal aberrations, sister chromatid exchanges, and micronuclei formations during investigations into nitrosamine-induced toxicity. (Glowienke et al., 2022; Phillipson & Ioannides, 1984)

Mitigation Strategies

The Food and Drug Administration has a three-step plan to deal with nitrosamine impurities.

1. The first step is to figure out the risk of nitrosamines in Active Pharmaceutical Ingredients and drug products that are already approved or waiting to be approved. The Food and Drug Administration wants companies to look at the risk and decide what to do and then keep records of what they find out in case the Food and Drug Administration needs to review them.
2. The second step is to do careful testing when a risk is found to make sure the results are accurate.
3. The third step is to tell the Food and Drug Administration and keep a record of any changes made to reduce or prevent nitrosamines and to do this in the way that the Food and Drug Administration says to.

Manufacturers of drug products should also make sure to include Nitrosamine Detection Studies and Risk Assessments in their risk assessments and to look at the risks again from time to time, as part of their management of nitrosamine impurities and the Food and Drug Administration regulations.

Control of Nitrite Introduced during Formulation

The level of nitrite in the formulation is a significant risk element in the production of NDSRIs,^{87,88} making it a timely target for introducing corrective and preventative actions (CAPAs). The current knowledge indicates that nitrite serves as the limiting reagent in the creation of NDSRIs, thereby influencing the maximum quantity of N-nitrosamine that can be produced in the drug formulation.³⁵ However, it's crucial to recognize that only a portion of the overall nitrite actually results in N-nitrosamine contaminants under typical formulation and storage situations, with the extent varying based on the reactivity of the susceptible secondary amine: generally, a few percent for aliphatic amines, while the nitrosation of aromatic derivatives can be significantly greater (for example, 25% nitrosation of N-methyl phenylamine).

Control of N-Nitrosamine Formation during Formulation

Instead of controlling nitrite in excipients, altering the formulation conditions could also help lower the risk of generating NDSRI. For example, nitrite scavengers could be added to the formulation; the existing list of candidates is extensive, and research in this area is progressing. Other regulators have expressed opinions on this strategy. Additionally, the pH of the formulation could be modified, either through a straightforward method like incorporating basic additives or, if suitable, by the more intensive replacement of salt forms of amine-containing APIs with their respective free bases. An additional area of focus could be the drug substance's physical attributes like polymorphism or particle dimensions; these aspects are recognized elements that affect solid-state NDSRI development.^{25,36} Modifying production methods by, for instance, changing the water content in the formulation could prove to be an effective strategy. Ultimately, steering clear of high-risk unit operations (such as fluid-bed drying and wet granulation) or modifying manufacturing process parameters may also lessen the formation of NDSRI during the formulation of drug products. (Cioc et al., 2023; Mergens & Newmark, 1980)

Molecules Capable of Inhibiting or Blocking N-nitrosamine Formation in Drug Products

Several classes of compounds have been shown to mitigate nitrosamine formation. (M. Homšak et al.) Reaction mechanisms have been proposed for three main classes: the redox pathway, through the diazotization of primary amines, and nitration reactions of phenols. (K.K. Nanda et al. and MG Simic et al.) Compounds capable of rapidly reducing the nitrosating agent to non-nitrosating nitric oxide (NO) can reduce or completely inhibit the formation of N-nitrosamine. This is achieved by acting as a competitive substrate for the nitrosating moiety. Both the absolute and relative concentrations of the nitrosating agent, amine and

blocking agent determine the effectiveness of the blocking agent on *N*-nitrosamine formation. (W.J. Mergens et al ..)

A wide range of potential nitrite scavengers are available for use as blocking agents in drug products. (M. Homšak et al.) Blocking agents may be water- or lipid-soluble. Water-soluble molecules include phenols, ascorbic acid, [sulfite](#), [bisulfite](#), and cysteine, although phenolics are not considered reliable blocking agents as they can facilitate transnitrosation. (W.J. Mergens et al ..)

Fat-soluble antioxidants that are currently used in drug products and that have nitrite scavenging ability include BHA, BHT and α -tocopherol. Recent data however indicate that the effectiveness of BHA and BHT might be limited. (M. Homšak et al.) Ingredients that are listed as “inactive ingredients” for approved drug products by the FDA are particularly suitable for formulation into drug products.

Table 5 : Nitrite scavengers for potential use in pharmaceutical solid oral dosage forms

Substance	Fat/water soluble	Oral forms on FDA Inactive Ingredient list
α -Tocopherol	Fat soluble	Capsule, solution, tablet, suspension
Ascorbic acid	Water soluble	Capsule, powder, solution, suspension, syrup, tablet
L-cysteine	Water soluble	Capsule, suspension, tablet
Glycine	Water soluble	Capsule, powder, solution, suspension, tablet
Arginine	Water soluble	Tablet
Lysine	Water soluble	Only intravenous forms available
Histidine	Water soluble	Capsule, suspension
Caffeic acid	Water soluble	-
Ferrulic acid	Water soluble	-
BHA	Fat soluble	Capsule, granule, solution, suspension, syrup, tablet
BHT	Fat soluble	Capsule, film, solution, tablet
PABA	Water soluble	-

(Bayne et al., 2023)

Future Direction

Future efforts to control nitrosamine impurities in pharmaceuticals will require a globally coordinated, multidisciplinary approach combining advanced analytical techniques, regulatory innovation, and formulation science. Priorities include developing ultra-sensitive methods like UHPLC-HRMS for detecting trace nitrosamines and gaining a deeper understanding of how formulation factors influence nitrosation. Regulatory frameworks must evolve to include predictive toxicology and harmonize AI limits across regions. Meanwhile, research into nitrite scavengers (e.g., ascorbic acid, cysteine, BHT) and their integration into drug formulations will continue, alongside advances in process analytical technology (PAT) for real-time monitoring. Ultimately, a shift toward preventive strategies such as redesigning synthetic pathways and using safer raw materials—will be essential for ensuring drug safety and regulatory compliance.

Conclusion

The presence of nitrosamine impurities in pharmaceutical products is a major public health issue because these substances are known to cause cancer and damage genetic material. This review explains that nitrosamines like NDMA and NDEA can come from various sources, including raw materials, intermediates,

solvents, catalysts, manufacturing processes, and storage conditions. Their formation usually happens when nitrosating agents interact with certain amines or amides in acidic environments. Multiple contamination cases, such as those involving sartans, ranitidine, metformin, and rifampicin, have led to global recalls and increased scrutiny from regulators. Improvements in analytical methods like LC-MS/MS, GC-MS, HRMS, and NMR have boosted our ability to find nitrosamines at very low levels. This supports better risk assessment and quality control. Regulatory agencies, including the FDA and EMA, have set acceptable intake (AI) limits and created three-step risk mitigation strategies. These strategies focus on identifying, confirming, and controlling nitrosamine impurities. Additionally, developing toxicity classification systems for NDSRIs offers a more nuanced way to categorize risk. Efforts to reduce these impurities are also growing at the formulation stage. This includes controlling nitrite levels and using nitrite scavengers like ascorbic acid, BHT, and α -tocopherol. However, their compatibility in formulations, long-term stability, and effectiveness are still being studied. Future control strategies will increasingly depend on incorporating predictive toxicology, understanding mechanisms, innovating processes, and using real-time monitoring with Process Analytical Technology (PAT).

In the end, protecting drug safety from nitrosamine impurities requires a proactive approach based on science and global cooperation. Ongoing collaboration among regulators, industry players, and scientific communities is essential to identify risks, improve detection methods, and implement strong preventive strategies, ensuring compliance with regulations and safeguarding patient safety in the changing pharmaceutical landscape.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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