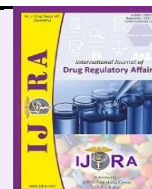


Available online on 15 Sep, 2026 at <https://ijdra.com/index.php/journal>**International Journal of Drug Regulatory Affairs**Published by Diva Enterprises Pvt. Ltd., New Delhi
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Review Article

Open  Access**Comparative Evaluation of Nitrosamine Impurity Control Strategies in USFDA, EMA, and WHO Pharmaceutical Submissions**Subalakshmi K ^{*,a}, Immaculate Sherlin Sheridan Vaz^a, Ramesh S^b^aDepartment of Pharmaceutical Regulatory Affairs, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai – 600116^bAssistant Professor, Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai – 600116**Abstract**

Nitrosamine impurities are of significant concern to the pharmaceutical industry because of their genotoxic and carcinogenic effects. They are formed during the manufacturing, storage, and/or packaging of APIs and/or drug products owing to the nitrosation reactions of amines with nitrosating reagents. The detection of nitrosamine impurities, such as N-nitrosodimethylamine and N-nitrosodiethylamine in medicinal products, including angiotensin II receptor blockers, ranitidine, and metformin, has raised significant concerns in the industry. Regulatory agencies have developed guidelines for controlling nitrosamine impurities. This review aims to evaluate the guidelines developed by the United States Food and Drug Administration, European Medicines Agency, and World Health Organization for controlling nitrosamine impurities and to identify the similarities and differences in the guidelines developed by the above agencies and the literature for risk evaluation, testing, and limits of intake, detection, and control of nitrosamine impurities. The review highlights the importance of strengthening international cooperation and developing standardised control strategies for the control of nitrosamine impurities in medicinal products, which is of significant importance for the quality and safety of medicinal products worldwide.

Conclusion: Effective control of nitrosamine impurities requires harmonized global guidelines, robust risk assessment, and advanced detection strategies to ensure the safety and quality of pharmaceutical products. Strengthening international collaboration is essential for developing consistent and reliable control measures worldwide.

Keywords: Nitrosamine impurities; Regulatory Affairs; acceptable intake limits; pharmaceutical contamination; USFDA; EMA; WHO; genotoxic impurities.

Article Info: Received 20 Apr 2026; Review Completed 17 Jul 2026; Accepted 20 Aug 2026**Cite this article as:**

Subalakshmi K, Sheridan Vaz IS, Ramesh S. Comparative Evaluation of Nitrosamine Impurity Control Strategies in USFDA, EMA, and WHO Pharmaceutical Submissions. *Int. J. Drug Reg. Affairs* [Internet]. 2026 Sep 15 [cited 2026 Sep 15]; 14(3):77-91. Available from: <http://ijdra.com/index.php/journal/article/view/905>

DOI: <https://doi.org/10.22270/ijdra.v14i3.905>

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1. Introduction

Nitrosamines are organic compounds characterised by the presence of a nitroso group attached to an amine group, as follows: $R_1N(R_2)-N=O$. They are derived from secondary or tertiary amines that react with nitrous acid via nitrosation. The International Agency for Research on Cancer (IARC) defines nitrosamines as mutagenic and carcinogenic impure substances and identifies them as N-nitroso compounds containing a -NNO or -NHNO group. (1,2) In 2018, millions of patients had to be recalled from valsartan products worldwide that had been contaminated by N-Nitroso dimethylamine (NDMA), which is a human carcinogen. However, short-term cancer risk assessment results indicated a minimal increase in risk. According to a study by Leclerc, manufacturers of generic valsartan products (pseudo-generics from Sandoz and Pro Doc from Canada) have been affected by changes in the

manufacturing of active ingredients since 2012. Contamination was accidentally discovered during non-routine testing. (3)

A Danish study in 2018 found a median follow-up of 4.6 years in 104 unexposed cancer patients and 198 NDMA exposed cancer patients with an adjusted hazard ratio of 1.09 (95% CI: 0.85-1.41) for overall cancer, showing no significant increase in short-term cancer risk, though it was found that there was a degree of uncertainty in cancer patients and it was recommended that longer follow-up studies should be conducted in the future. (4) Ranitidine formulations have been found to contain NDMA as an impurity; therefore, these formulations were recalled and removed from the market in various parts of the world in 2020. The United States Food and Drug Administration (FDA) fixed the amount of NDMA per dose at 96 ng because NDMA is formed during the storage of ranitidine

at increased temperature and humidity and in the simulated gastric environment with the presence of nitrites. (5-8)

Although, in the epidemiological study conducted in humans, mixed results were observed, and in one study conducted in South Korea, no significant increase in overall risk of cancer was observed in comparison with the famotidine group (7.45 vs. 7.56) ($p=0.716$). (9) However, in the study conducted by Braunstein, an association was observed between ranitidine and breast, thyroid, bladder, and prostate cancer, and the study was limited to the application in oncology only. NDMA was successfully detected in metformin products, and contamination was observed in a significant but lesser percentage of metformin products, i.e., 17.8%, at levels above 96 ng/day, and in an independent study on 105 tablets of metformin, NDMA was observed in 85.3% of metformin products, and 28 patients were above the limits set by European agencies. (10,11)

Several analytical techniques have been developed and validated, including GC-MS/MS, LC-MS/MS, and high-resolution mass spectrometry, with reported findings that N,N-dimethylformamide (DMF) interferes with NDMA analysis; therefore, analytical selectivity in the analysis of these compounds is essential. These cases of contamination have raised serious concerns in the international community of regulators regarding product recalls. (12,13) Nitrosamines are common environmental human compounds with a wide range of applications in foodstuffs, tobacco products, pharmaceuticals, occupational settings, and environmental carcinogens, with increasingly reported carcinogenic activities in various species and scenarios. (14-16) The carcinogenicity of nitrosamines in various animal studies has been reported, with these compounds inducing tumours in various animal species, including higher primates, which resemble human subjects in both biochemical and morphological characteristics. These compounds are etiological risk factors in various human cancers, including stomach, oesophageal, and nasopharyngeal tumours. (17)

Nitrosamines are genotoxic carcinogens that cause DNA damage through metabolic activation, and it has been reported that not all nitrosamines have similar potency as carcinogenic agents, with a significant difference between the drugs in this category. (18,19) Recently, contamination of commonly used drugs has raised global concern, and regulatory agencies worldwide are looking into the matter, resulting in the recall of the affected products. Nitrosamines are unintentionally formed during synthesis, storage, and sometimes through excipients and containers of the drugs, and it has been reported that a nanogramme per day exposure over a period of 70 years can cause cancer; hence, worldwide agencies have ordered the manufacturers of the affected products to conduct risk assessments for nitrosamines and ensure that the levels are kept below the acceptable levels for each compound. (20-23)

The control of nitrosamines is important during the development of active pharmaceutical ingredients (APIs), drug product development, and marketing authorisation applications because of safety concerns. Nitrosamines are probable human carcinogens with genotoxic properties.

They are a global safety concern in the pharmaceutical industry. (24, 25) In the development of APIs, nitrosamines are produced through the reaction of secondary/tertiary amines and nitrites. It is important to evaluate the synthesis route and materials. (26, 27) API manufacturers are responsible for developing a method for the detection of nitrosamine impurities using GC-MS or LC-MS/MS techniques to evaluate product quality. (28, 29) In drug product development, nitrosamine formation occurs during the manufacturing process through the reaction of nitrites and amine-containing APIs or excipients. Excipients with low nitrite levels are important in controlling the risk of contamination. Research indicates that replacing excipients is effective in controlling the formation of nitrosamines to levels required by intake limits. (30)

Comprehensive risk assessments are required for nitrosamines in marketing authorisation submissions. The presence of nitrosamines in ARBs, ranitidine, and metformin has led to guidelines that include risk assessments on the sources of nitrosamines. Marketing authorisation holders are required to provide analytical data and risk mitigation strategies. (31) Nitrosamine impurities in drugs are a major concern for regulatory authorities. Nitrosamine impurities are known to be carcinogenic. Following the detection of nitrosamine impurities in various drugs, such as angiotensin II receptor blockers, ranitidine, and metformin, stringent guidelines were issued by the authorities. Although international agencies are working toward a common aim to ensure patient safety, there are differences in how each agency is working toward this aim. This review is aimed at understanding the regulatory framework issued by USFDA, EMA, and WHO on how to manage nitrosamine impurities. This review aims to understand the rationale for regulatory guidelines on managing nitrosamine impurities.

2. Chemistry and Structural Characteristics of Nitrosamine Impurities

Nitrosamides are a class of nitroso compounds characterised by unique physicochemical properties, such as molecular weight, boiling point, solubility, and volatility. Nitrosamides have unique molecular weights. The molecular weight is unique to each class of compound and is related to its structure or functional group. Molecular weight is an important parameter because it affects the diffusion, bioavailability, and interaction of a compound with biological membranes. (32) The boiling point is related to the molecular weight and is characterised by unique boiling points for each class of compound. Generally, the boiling point is related to the molecular weight such that as the molecular weight increases, the boiling point also increases. Nitrosamides have unique boiling points due to their structure or functional groups. (33, 34)

The solubility of nitrosamides varies depending on polarity, structure, and functional groups. Most nitrosamides have low water solubility because of their polarity. However, nitrosamides may have good solubility in organic solvents. These characteristics of nitrosamides are essential in pharmaceutical applications because of

their formulation, stability, and use in drug delivery systems. Volatility of nitrosamides: This is an important characteristic of nitrosamides. Nitrosamides may be volatile or semi-volatile. The most volatile nitrosamides have a low molecular weight and sufficient vapour pressure to be easily vaporised. These characteristics of volatile nitrosamides are essential in pharmaceutical applications because of the potential inhalation hazard of these compounds. (35) These characteristics of nitrosamides, including molecular weight affecting the size of the molecule and its diffusion, boiling point affecting phase changes in the molecule, solubility affecting compatibility with mediums, and volatility affecting the molecule in analysis and use, are essential in understanding the role of nitrosamides in pharmaceutical applications.

Table 1. Common nitrosamine impurities (37)

Nitrosamine	Abbreviation	Molecular Formula
N-Nitrosodimethylamine	NDMA	C ₂ H ₆ N ₂ O
N-Nitrosodiethylamine	NDEA	C ₄ H ₁₀ N ₂ O
N-Nitrosodiisopropylamine	NDIPA	C ₆ H ₁₄ N ₂ O
N-Nitrosoethylisopropylamine	NEIPA	C ₅ H ₁₂ N ₂ O
N-Nitroso-N-methyl-4-aminobutyric acid	NMBA	C ₅ H ₁₀ N ₂ O ₂

Nitrosamines are classified according to their volatility into two major groups:

Volatile nitrosamines: These include low-molecular-weight nitrosamines with sufficient vapour pressure for vaporisation. Examples are:

- NDMA (N-nitrosodimethylamine): A small, volatile, polar nitrosamine that is found as a disinfection byproduct and pharmaceutical impurity. It is water-soluble and has been detected using gas chromatographic methods. (38,39)
- NDEA (N-nitrosodiethylamine): Larger and less volatile than NDMA but still volatile, soluble in organic solvents, and a probable carcinogen found at industrial and environmental sources. (39)

Non-volatile nitrosamines: These are higher molecular weight or cyclic nitrosamines with lower vapour pressures and persistence. Examples include:

- NPIP (N-nitrosopiperidine): A cyclic nitrosamine with carcinogenic effects, formed during pharmaceutical manufacturing and food processing. It has low volatility due to its ring structure. (40)
- NDIPA (N-nitrodipropylamine): A nitrosamine from dipropylamine, which is less common and has lipophilic properties and carcinogenic potential similar to other non-volatile nitrosamines.

The volatile nature of nitrosamines, such as NDMA and NDEA, creates problems in analysis and safety during use in both vapour and aqueous solutions. In contrast, non-volatile nitrosamines have a longer life in formulations and require consideration of their presence and toxicity risks.

3. Sources and Formation Mechanisms of Nitrosamine Impurities

3.1 Nitrosation Reaction Mechanism

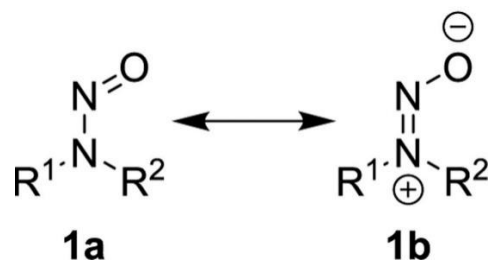


Figure 1. Two major resonance contributors to nitrosamines. (36)

Although nitrosamines are typically depicted as structure 1a, their actual structures and chemical reactivity are heavily influenced by the zwitterionic resonance structure 1b

The reaction of these amines with nitrosating agents results in the production of N-nitrosamines. These compounds are of interest in toxicity studies because of their known carcinogenic activity. Secondary amines react with nitrosating agents, such as nitrous acid, which is produced in an acidic environment from nitrites. These reactants form N-nitrosamines. The reaction of secondary amines with nitrosating agents occurs through an electrophilic attack of the nitrosonium ion (NO⁺) or dinitrogen trioxide (N₂O₃) on the nitrogen of the secondary amine. The reaction rate of these amines with nitrosating agents depends on the pH of the solution. It is known that an acidic environment with a pH of approximately 1-3 enhances the reaction rate. The catalysis of the reaction using ions, such as thiocyanate, in an acidic environment simulates the gastric environment. (41-43)

Tertiary amines are not directly involved in nitrosation to yield N-nitrosamines because they do not possess a second hydrogen on the nitrogen. However, these compounds are involved in complex reactions, such as nitrosative dealkylation, to yield secondary amines, which are subsequently involved in the formation of N-nitrosamines. This process is 1000 times slower for tertiary amines. Some functional groups are involved in facilitating resonance or inductive effects in tertiary amines. Nitrosation of tertiary amines is catalysed under certain conditions. Tertiary amines such as piperazine are involved in the formation of nitrosamines under certain industrial processes. (44,45)

The major nitrosating agents are derived from nitrites and nitrates. Nitrites are more active under acidic conditions. They produce nitrous acid upon reaction with an acid. Nitrous acid is then converted into nitrosonium ions and dinitrogen trioxide. The nitrosyl compounds, which are derived from NO⁺ and N₂O₃, are electrophilic nitrosating agents that react with amines. The nitrosamine reaction is affected by environmental and biological factors, such as

pH, catalysts, such as thiocyanate ions, and the chemical environment, such as micelles or carbonyl compounds (42, 43, 46, 47). The reaction between secondary/tertiary amines and nitrosating agents is a complex nitrosation reaction that involves the nitrosation of secondary amines and various nitrosation reactions for tertiary amines. The kinetics of this reaction are affected by environmental factors and catalysts.

3.2 Potential Sources in Pharmaceutical Manufacturing

In pharmaceutical manufacturing, potential sources of nitrosamine include several components and process factors.

- **API Manufacturing:** Amine-containing intermediates in API synthesis can serve as nitrosamine precursors. Solvent degradation can release amines; for example, dimethylformamide (DMF) degradation yields dimethylamine, a secondary amine that is susceptible to nitrosation.
- **Excipients:** Some excipients contribute to nitrosamine contamination. Notably, crospovidone and sodium starch glycolate are potential sources of contamination because of their chemical composition or risk of contamination during processing.
- **Reagents and Catalysts:** Nitrite-containing reagents are significant sources of nitrosating agents. Under appropriate conditions (acidic pH and the presence of amines), nitrites can facilitate nitrosation reactions, resulting in the formation of nitrosamines.
- **Packaging Materials:** Components in packaging, especially rubber materials, can introduce nitrosamine contamination. Rubber materials may contain nitrosating agents or amine precursors that migrate into drug products, raising safety concerns. (45)

Influence of environmental and process factors on nitrosamine formation during manufacture and storage

- **pH:** Acidic conditions favour nitrosation, enhancing nitrosamine formation from amines and nitrosating agents.
- **Temperature:** Elevated temperatures can accelerate nitrosamine generation during processing and storage.
- **Oxygen:** Oxygen can have catalytic or oxidative effects that influence nitrosamine formation pathways. (46-48)
- **Storage Conditions:** Temperature, humidity, and exposure to reactive substances affect nitrosamine stability and the risk of its formation. (49,50)

Knowledge of these sources and environmental factors is important in the manufacture of pharmaceuticals to minimise the risk of nitrosamine formation in drug products. Risk assessment and control of the risk factors of precursors, nitrosating agents, excipients, and packaging materials minimize the risk of the formation of nitrosamines.

4. Occurrence of Nitrosamine Impurities in Pharmaceutical Products

4.1 Angiotensin II Receptor Blockers (Sartans)

The nitrosamine contamination of angiotensin II receptor blockers that contain tetrazole rings has been related to synthetic routes utilised during the formation of the tetrazole moiety. The tetrazole ring is synthesised using azide chemistry and nitrile compounds. Sodium azide is utilised as a reagent to convert nitrile functional groups into a tetrazole ring. Impurities that contain nitrogen are introduced during the synthetic route, which is utilised to form nitrosamines. Secondary amines, solvents, or reagents utilised during the synthetic route can react with nitrites to form carcinogenic nitrosamines, such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). (51,52) Improper conditions of synthesis during the formation of the tetrazole ring, the presence of nitrite in the reagents, poor control of pH and temperature, and poor removal of amines can lead to the presence of nitrosating species and the formation of nitrosamines.

Changes in the manufacturing process without risk assessment of the risk of nitrosamine formation have been implicated in contamination incidents that have been reported globally. The process of contamination depends on the tetrazole synthesis, which utilises nitrogen-rich reagents that may aid nitrosation reaction pathways. Consequently, regulatory attention and analysis of nitrosamine impurities in tetrazole-containing sartans have been conducted using sophisticated analytical techniques, such as headspace gas chromatography–mass spectrometry, at trace levels. The presence of nitrosamines as impurities in sartans resulting from the synthesis of the tetrazole ring is due to the nitrosation reactions between the amine and the nitrosating agents in the conversion of the azide/nitrile to the tetrazole.

4.2 Ranitidine and Nizatidine

H₂ receptor antagonists' ranitidine and nizatidine degrade to N-nitrosodimethylamine (NDMA), a probable human carcinogen. Drug degradation is mainly due to thermal degradation and storage. Forced thermal degradation studies on ranitidine and its impurities revealed that the drug and its impurities degraded to NDMA at different rates. Some impurities degraded to 100 times more NDMA than the drug at 110°C. (53) Storage conditions also impact NDMA formation. Ranitidine tablets stored under accelerated conditions (40 °C and 75% relative humidity) showed an increase in NDMA content over a period of weeks, beyond the acceptable intake levels. Factors such as moisture, oxygen, and nitrite levels impact NDMA formation, and these are environmental factors. (54)

The dimethylamine group present in ranitidine is liable to classical nitrosating reactions with sources of nitrite to yield NDMA. In vitro experiments demonstrated that nitrosation reactions between ranitidine and sodium nitrite yield NDMA. Antioxidants, such as ascorbic acid and trolox, can inhibit these reactions to some degree. (55) Chloramination reactions involving ranitidine contribute to NDMA formation through reactions involving chloramine species. The dichloramine reacts with the ranitidine structure at a high yield to produce NDMA. Chlorinated and hydroxylated species are formed as part of the reaction mechanism. The carbocations of

methylfurans are also involved in the reaction mechanism. (56,57)

Similar concerns exist for nizatidine. Nizatidine shares structural similarities with ranitidine, including the tertiary amine group, which is capable of NDMA formation. “Ultra high-pressure liquid chromatography coupled with triple quadrupole mass spectrometry approaches have detected NDMA in nizatidine active pharmaceutical ingredients and finished dosage forms. (58) Ranitidine and nizatidine degrade to NDMA via thermal degradation, nitrosation with nitrite and chloramine, and storage under humid and warm conditions. Monitoring of impurities, storage, and nitrosation inhibitors is important in controlling NDMA contamination risk.

4.3 Metformin Products

Nitrosation reactions that occur during metformin production may result in the presence of nitrosamine compounds that act as potential carcinogens and have safety implications in the field of pharmacy. Nitrosamines may be formed as a result of the interaction between amine compounds and nitrosating agents, which include compounds containing nitrite during drug manufacturing. (59) Nitrosation of metformin occurs through a reaction of secondary or tertiary amine groups with nitrosating agents under acidic conditions, in which nitrous acid is generated from nitrites. The nitrosation rate may be affected by the pharmaceutical vehicle, catalysts, solvents, or excipients; nitrite contamination of raw materials can enhance impurity formation.

Complex nitrosamine drug substance-related impurities can form through alternative mechanisms involving the reaction of the active pharmaceutical ingredient with nitrosating agents during processing. Analytical techniques play a crucial role in the identification of ultra-trace levels of impurities in metformin using liquid chromatography tandem mass spectrometry techniques because of the potential of these impurities at ultra-trace levels. (60) Control of nitrosating agents, prevention of nitrite contamination, monitoring of formulation pH and

reaction conditions, and routine impurity testing are critical to minimising nitrosamine impurities in metformin manufacturing.

4.4 Other Drugs

Nitrosamines have been identified in various pharmaceutical compounds, including rifampicin, varenicline, and propranolol, thus posing a risk to the safety of these compounds, particularly with regard to carcinogenicity. Rifampicin, a medication used to treat tuberculosis, is a pharmaceutical agent in which the risk of nitrosamines is a concern. Although the specific mechanism underlying the presence of nitrosamines in rifampicin has not been well documented, in other compounds, this risk is attributed to the amine groups, which may react with nitrosating agents during the synthesis, formulation, and storage of the active pharmaceutical ingredient (API). Another drug in the category of smoking cessation agents, varenicline, also contains nitrosamine impurities. These impurities arise from the reaction of secondary or tertiary amine groups with nitrosating agents that may be present as a result of the reagents or process conditions used in drug manufacture.

Propranolol is a beta-blocker used to treat hypertension and other cardiovascular diseases. However, it has the potential to contain nitrosamines as impurities. The presence of aliphatic amines makes it prone to nitrosation if nitrites are present during its manufacturing or storage. Nitrosamine impurities are usually derived from nitrosation reactions with amine groups and nitrosating agents, which may be present as impurities in raw materials, solvents, or formed as by-products under acidic conditions. The complexity of manufacture requires risk assessment, control of nitrosating agents, and the use of sensitive techniques for detection of nitrosamine impurities for regulatory compliance. Rifampicin, varenicline, and propranolol carry the risk of nitrosamine impurities as part of a wider problem affecting pharmaceuticals, requiring strict monitoring throughout the manufacturing and supply chain.

Table 2. Comparison of drug classes with nitrosamine impurities

Drug Class	Common Nitrosamine Impurities
Angiotensin II Receptor Blockers (Sartans)	NDMA (N-Nitrosodimethylamine), NDEA (N-Nitrosodiethylamine), NDIPA (N-Nitroso-Diisopropylamine), NEIPA (N-Nitrosoethylisopropylamine), NMBA (N-Nitroso-N-methyl-4-aminobutyric acid)
Histamine-2 Receptor Blockers (e.g., Ranitidine, Nizatidine)	NDMA
Antidiabetic Drugs (e.g., Metformin, Pioglitazone)	NDMA and other related N-nitrosamines from nitrosation reactions
Antibiotics (e.g., Rifampicin)	Various nitrosamines reported, specific compounds less documented but known risk due to amine groups
Smoking Cessation Agents (e.g., Varenicline)	N-nitroso derivatives detected due to amine functionalities and manufacturing conditions
Beta-Blockers (e.g., Propranolol)	Nitrosamine impurities from nitrosation of amine groups

The above table indicates that nitrosamines such as NDMA and NDEA are commonly found in many classes of pharmaceuticals, particularly those with secondary and tertiary amines that are susceptible to nitrosation. This is due to the risk of nitrosation from the manufacturing process, reagents, excipients, and storage, which may

contain nitrosating agents and promote the formation of nitrosamines. The detection and control of these impurities require the application of sophisticated analytical techniques, such as LC-MS, GC-MS, and ultra-sensitive methods, as per the guidelines to ensure patient safety and compliance.

5. Analytical Detection Methods for Nitrosamine Impurities

5.1 Chromatographic Techniques

Gas chromatography–mass spectrometry (GC-MS) is a key analytical technique for the selective detection of nitrosamine impurities in pharmaceutical formulations. Nitrosamines are semi-volatile compounds with varying polarity and considerable toxicological significance. These require selective analytical techniques for their detection at trace levels owing to their genotoxic and carcinogenic properties. The GC-MS system has the advantage of uniting the sensitivity of gas chromatography to separate volatile and semi-volatile nitrosamines according to their physicochemical properties with the sensitivity and specificity of mass spectrometry for particular ion fragments according to their mass-to-charge ratio. Using this system, nitrosamines, such as N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), and related derivatives have been detected. This approach employs sample preparation techniques, such as solid-phase extraction or headspace analysis, to pre-concentrate the analytes and minimise matrix effects.

Headspace gas chromatography with nitrogen phosphorus detection (NPD) increases the selectivity for nitrogen-containing nitrosamines, whereas full-evaporation static headspace techniques increase quantification to sub-ppb levels, exceeding liquid chromatography capabilities. The prevention of in situ nitrosation artefacts in GC analysis, including the addition of antioxidants such as pyrogallol and the use of solvents such as phosphoric acid and isopropanol, helps prevent false positives. GC-MS methods have proven their efficacy in meeting international regulations, such as ICH Q2(R1), ensuring precision, accuracy, and ruggedness in the routine quality control of active pharmaceutical ingredients and drug products. GC-MS analysis of impurities in drug substances in the form of nitrosamines cannot be replaced with any other method because of its importance in ensuring drug safety and regulatory compliance worldwide.

LC-MS/MS is a commonly employed chromatographic method that is utilised for the sensitive detection of nitrosamine-related impurities in pharmaceutical products. The carcinogenic potential of nitrosamines has led various regulatory bodies to demand sensitive detection of trace-level nitrosamine-related impurities within pharmaceutical products with a high degree of precision. LC-MS/MS is a powerful tool that offers a combination of chromatographic resolution along with the high sensitivity and specificity of tandem mass spectrometry. LC-MS/MS has been employed for the identification and quantification of nitrosamines, such as N-nitroso fluoxetine, N-nitroso dabigatran etexilate, and N-nitroso dibutylamine, at extremely low detection limits in parts per billion in pharmaceutical products. Optimisation of chromatographic parameters, such as C18 columns, mobile phases that contain buffers such as ammonium acetate or formic acid with pH adjustments, flow rate, and gradient elution, is a significant step that is incorporated during method development. In addition, electrospray ionisation in the positive mode coupled with multiple

reaction monitoring is employed for the sensitive detection of nitrosamines.

The validation parameters are based on international guidelines, such as ICH Q2(R1), which include the evaluation of linearity, detection limits, precision, accuracy, and specificity. The detection limits are in the range of sub-ppb to low ppb levels. The recovery rates are between 80% and 120%, and the correlation coefficients are >0.99 for the calibration curves over the concentration ranges. These results confirm the quantification of trace amounts of the drug in finished products and APIs. The LC-MS/MS method can simultaneously quantify multiple nitrosamine impurities, including the newly identified NDSRIs. This method provides a comprehensive profile of the impurities present in the drug. Sample preparation techniques include solid-phase extraction and liquid-liquid extraction. The LC-MS/MS technique plays a critical role in the analysis of nitrosamine impurities because it is a sensitive and specific technique that can easily handle complex samples. It is the best technique for a regulatory compliant approach to drug safety.

Chromatographic techniques coupled with high-resolution mass spectrometry (HRMS) have been established as efficient tools for the detection and quantification of trace levels of nitrosamine impurities in pharmaceuticals. Nitrosamines, because of their genotoxic and carcinogenic properties, demand sensitive and specific detection and quantification at trace levels. Liquid chromatography (LC) is recommended for the analysis of polar and thermally unstable nitrosamines. HPLC and UHPLC techniques using C18 columns have been recommended for the analysis of nitrosamines. Aqueous mobile phases containing ammonium acetate buffer and organic modifiers, such as methanol and acetonitrile, have been recommended. HRMS analysis provides accurate mass measurements of the analytes with a mass accuracy of within 5 ppm. This accuracy is essential for the identification and quantification of multiple nitrosamine impurities in complex matrices. It also helps differentiate between analytes and interferences. HRMS analysis provides the advantage of detecting trace levels of analytes, as per regulatory requirements.

Sample preparation techniques, such as SPE and LLE, are employed to enrich nitrosamines. These techniques enhance the sensitivity and specificity of analytical methods. Method development involves optimising the gradients and MS conditions, including the ionisation mode and fragmentation energy, to enhance the performance of the analytical method. This helps to enhance the performance of the analytical method. Chromatography HRMS is a technique that combines the power of chromatography with the sensitivity of HRMS. This technique provides a robust and sensitive method for the impurity profile of nitrosamines in pharmaceutical products. Chromatography techniques are employed for the detection of nitrosamine impurities in pharmaceutical products. High-performance liquid chromatography with ultraviolet detection is employed for the detection of nitrosamine impurities. This technique is efficient for detecting nitrosamines. It is employed for the quality control of nitrosamine impurities in pharmaceutical products.

The genotoxic and carcinogenic impurities in drug substances, nitrosamines, can be separated using reversed-phase high-performance liquid chromatography (HPLC) with UV detection. In a developed LC-MS method, reversed-phase HPLC with a C18 column and UV detection was used to separate major nitrosamine impurities, including NDMA, NDEA, NMEA, NDIPA, NDMA, and NPIP, with good resolution and sensitivity. A methanol and 0.1% formic acid in ultrapure water mobile phase was used with gradient elution and separation at mild temperatures and moderate flow rates, with a separation time of less than 25 min. HPLC-UV methods used for the detection of nitrosamines are easy to use and cost-effective, especially in cases where MS cannot be used. HPLC with UV detection of drug substances and assay impurities, including nitrosamines, can be detected based on their UV absorption. These methods are reliable in drug substance studies, including drug dissolution and impurity profiling, as well as enantiomeric separations.

Highly sensitive requirements for the detection of nitrosamine impurities have prompted the development of ultra-sensitive chromatographic techniques that use HPLC coupled with MS/MS detection. HPLC-UV is also an important initial method that is sufficiently sensitive if the conditions are optimised. Chromatographic techniques using reversed-phase columns containing mobile-phase buffers and gradient programs allow the simultaneous determination of several nitrosamines. The method also demonstrates sufficient specificity, precision, and linearity to meet regulatory requirements. HPLC-UV techniques use reversed-phase columns and gradient elution programs containing organic mobile phases and acidic buffers. UV detection provides selectivity for the detection of nitrosamine impurities. HPLC-UV provides a reliable and efficient analytical method for the detection of impurities. Coupling the technique with mass spectrometry provides ultra-sensitivity for the detection of trace levels of impurities at the ppb level.

5.2 Sample Preparation Techniques

Sample preparation techniques are critical for detecting impurities, such as nitrosamines. This is because matrix effects from pharmaceutical or biological samples may cause inaccurate data. Techniques such as solid-liquid extraction (SLE), liquid-liquid extraction (LLE), and solid-phase extraction (SPE) are applied to remove impurities from samples. In SLE, a liquid solvent is used to extract impurities from a solid sample by penetrating the matrix. Superheated liquid extraction (SHLE) is an advanced technique used to improve SLE by raising the temperature and/or pressure below the critical point of the solvent to increase the penetration and solubility of impurities from solid pharmaceutical samples.

Liquid-liquid extraction (LLE) involves the partitioning of analytes between two immiscible liquids. During LLE, the analytes of interest partition into the organic phase. This helps to separate the analytes from the aqueous sample matrix. However, LLE is a labour-intensive method that also faces challenges, such as the formation of emulsions and the use of organic solvents that may be harmful. LLE requires large volumes of the sample and is likely to co-extract interfering compounds from the sample matrix. In

contrast, solid-phase extraction (SPE) is a selective method for extracting the analytes of interest. During SPE, the sample is passed through a solid-phase extraction cartridge. Analytes of interest are retained on the stationary phase of the SPE cartridge. Interfering compounds in the sample are washed out of the SPE cartridge. SPE is an efficient method for extracting the analytes of interest, as it uses less solvent. SPE is also an efficient method for extracting the analytes of interest, as it uses less solvent.

It is often employed in conjunction with chromatographic techniques to improve sensitivity. Sample preparation is a key step in the analytical procedure. It is important because matrix components may interfere with the result. Hence, the method of extraction must be able to maximise the recovery of nitrosamines without any interference. New techniques, such as solid-phase micro-extraction, which combines the processes of extraction and concentration with a reduced amount of solvent consumption, are time-saving and minimise the effect of the matrix. Solid-liquid extraction is best for the extraction of nitrosamines from solid samples. However, liquid-liquid extraction is based on the partitioning principle and exhibits the problem of emulsions. Solid-phase extraction provides the advantage of reduced interference. Sample preparation techniques are important for the accurate detection of nitrosamine impurities.

5.3 Sensitivity Requirements

The sensitivity parameters in the identification of nitrosamine impurities in pharmaceuticals are critical for ensuring the safety of pharmaceutical products because of the known carcinogenic activity of these impurities. These parameters include the limit of detection (LOD) and limit of quantification (LOQ). The LOD refers to the lowest concentration of an analyte that can be detected using an analytical method, whereas the LOQ refers to the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable accuracy and precision. Using an LC-MS/MS method to identify impurities in pharmaceuticals, a LOD as low as 0.03 µg/mL or ppb levels has been attained in the identification of nitrosamine impurities in pharmaceuticals. Similarly, the LOQ of an LC-MS/MS method in the identification of impurities in pharmaceuticals ranges from low ng/mL or ppb levels, including 0.08 µg/mL or 0.004 ppm in the identification of multiple nitrosamines.

The analytical methods must be approved by agencies such as the US FDA, EMA, and ICH for the identification and determination of these impurities at levels below the Acceptable Intake limits for safety. As nitrosamines are genotoxic carcinogens at trace levels, higher sensitivity levels are required. LC-MS/MS methods for nitrosamines, such as NDMA and NDEA, have LOD and LOQ levels well below the acceptable limits, allowing for accurate monitoring and determination of drug substances and products. These methods must be linear with a correlation coefficient > 0.99, precise, accurate with recovery rates between 80-120%, and specific to avoid interference from the matrix. These methods must comply with ICH Q2(R1) and meet pharmacopoeial standards for the method's fitness for purpose in quality control and regulatory

submissions. Sensitivity levels for these analytical methods are based on the requirement that LOD and LOQ levels should be at trace levels, well below the acceptable limits. LC-MS/MS methods meet these requirements for sensitivity with high specificity, accuracy, and reproducibility.

6. Global Regulatory Framework for Nitrosamine Control

The international framework on the control of nitrosamines has evolved from being scientifically driven to policy-driven to ensure public health protection. This is because regulators have recognised the carcinogenic and genotoxic risks associated with nitrosamines, necessitating effective strategies for detecting and assessing these risks in pharmaceutical production processes. For instance, after recognising the carcinogenic risk associated with nitrosamines, these impurities were identified in commonly used drugs, such as angiotensin receptor blockers (valsartan), ranitidine, and metformin. This led to a global recall of these drugs. As a result, regulatory authorities such as the USFDA, EMA, and Health Canada (HC) stepped up their surveillance activities to set guidelines on how to control nitrosamine impurities.

The framework for controlling nitrosamine impurities requires pharmaceutical companies to assess APIs and finished products to identify possible contamination with these impurities. This is done by considering various factors, such as the production process, storage conditions, and presence of nitrosating agents. In addition, pharmaceutical companies are required to use strategies, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), to determine the concentration of these impurities in finished products to ensure that they are within permissible limits.

Regulators help in this process through the development and harmonisation of acceptable intake (AI) limits using methods such as the Carcinogenic Potency Categorisation Approach (CPCA), which categorises nitrosamines based on their predicted carcinogenic potencies. The CPCA helps in developing AI limits in cases where specific compound-related empirical data are unavailable. It ensures the development of safety limits based on science and international agreement. Global regulatory activities include reviewing and communicating risks, as illustrated in the recent EMA audit of various nitrosamine-contaminated products, resulting in harmonised guidance on process optimisation. It results in harmonized guidance in process optimization. Communication between industry and regulatory authorities helps in finding solutions based on science and balances patient safety with medicine supply. This demonstrates how regulatory affairs professionals use toxicological and analytical data to develop standards, submissions, and surveillance activities aimed at ensuring pharmaceutical quality globally. It has transformed from understanding the nature of nitrosamines in pharmaceuticals to risk-based regulations using various analytical techniques and ensuring compliance with acceptable intake limits in an effort to address risks and ensure patient access to safe medicine.

6.1 Global recalls

International regulatory actions on nitrosamine impurities in pharmaceuticals have set off a chain reaction of product recalls worldwide, focused on identifying impurities in pharmaceuticals. The presence of nitrosamine impurities in drugs, such as valsartan, ranitidine, and metformin, has led to immediate safety announcements and recalls to prevent patient exposure to these known carcinogens. Following the detection of N-nitrosodimethylamine (NDMA), a known nitrosamine impurity, in valsartan drugs by certain manufacturers in 2018, the U.S. The Food and Drug Administration (USFDA) and other international authorities issued product recalls and safety announcements in the U.S., Europe, and Canada to investigate the manufacturing processes and identify routes for the formation of these impurities. Similarly, product recalls were issued for ranitidine and metformin drugs after these impurities were detected, reflecting the emphasis on patient safety in light of emerging safety data.

These recalls were initiated to withdraw contaminated products and prompted regulatory agencies to establish guidelines on risk evaluation, control strategies, and development of analytical methods for the determination of nitrosamine impurities. The combined efforts of the international community have provided drug product manufacturers with acceptable limits of intake and recommendations for the determination of impurities using techniques such as LC-MS/MS. This has also prompted the development of more effective detection strategies. The recalls initiated by the regulatory agencies across the globe emphasize the need to address the genotoxic impurities.

6.2 Risk assessment requirement

The risk assessment process is at the core of the international regulatory framework for controlling nitrosamine-related impurities in pharmaceutical products. Since 2018, following the detection of nitrosamine-related impurities in pharmaceutical products, including angiotensin II receptor blockers, ranitidine, nizatidine, and metformin, regulatory agencies such as the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and Health Canada have mandated that pharmaceutical companies conduct detailed risk assessments of pharmaceutical products that have the potential to be affected by nitrosamine-related impurities. This includes assessing all active pharmaceutical ingredients, excipients, and manufacturing processes, including pharmaceutical products, that have the potential to be affected by nitrosamine-related impurities. It also involves evaluating all processes and conditions that could lead to the generation of nitrosamine-related impurities, including synthetic processes, raw materials, and storage conditions. Consequently, pharmaceutical companies are required to conduct detailed risk assessments of all pre-approved and approved pharmaceutical products, including those that have been on the market before 2018, and have prioritised these assessments based on factors, such as maximum daily dose, duration of therapy, therapeutic indication, and patient population size. This is because nitrosamines are potent genotoxic carcinogens and, therefore, must be within an acceptable intake (AI) limit.

The industry utilises sensitive analytical techniques to detect and quantify these impurities at trace levels and employs toxicological extrapolations based on available empirical evidence. Collaboration between industry and regulatory agencies helps improve these techniques, resulting in a series of control strategies with a focus on patient risk assessment. Risk assessment should be conducted prospectively during drug development and retrospectively for marketed products. The evaluation of potential nitrosamine impurities from various sources, including active pharmaceutical ingredients, excipients, equipment used in the process, and packaging materials, should be systematic. If nitrosamines or their precursors are inevitable in the process, various controls may be necessary. Regulatory agencies have mandated a thorough risk-based assessment of the nitrosamine contamination potential in the pharmaceutical industry. These risk assessment techniques are essential in ensuring the impurities remain at acceptable levels and in complying with an ever-evolving regulatory environment with a focus on proactive impurity control.

6.3 Post-approval monitoring

This is an important aspect of the global regulatory framework for nitrosamine control. Post-approval monitoring is crucial to ensure the safety and quality of pharmaceutical products. Regulatory authorities mandate continuous post-approval monitoring of drug substances to ensure that the drug product does not contain impurities in excess of acceptable intake (AI) limits. Corrective measures should be adopted in such situations. Post-approval monitoring involves testing drug substances and finished drug products using sensitive analytical techniques, such as liquid chromatography tandem mass spectrometry (LC-MS/MS), to identify impurities containing nitrosamines and drug substance-related impurities (NDSRIs), particularly those with carcinogenic and genotoxic activities. Pharmacovigilance activities are highlighted in various guidelines.

This post-approval monitoring is useful to identify any possible new sources for nitrosamine formation that are associated with changes in processes, raw materials, packaging materials, or storage conditions. Regulatory authorities expect pharmaceutical companies to take measures to control these factors and communicate with health authorities to avoid patient risk. Additionally, the regulatory environment promotes communication between industry and health authorities to discuss patient health risks, improve existing methods to analyse data, and improve post-approval monitoring strategies based on available data and technology to ensure the supply of safe medicines according to globally harmonised standards. Post-approval monitoring in the global nitrosamine regulatory environment involves pharmaceutical companies in conducting analytical testing, re-evaluating risk, and maintaining impurity control during product commercialization to ensure patient health and safety.

7. USFDA Regulatory Requirements

7.1 FDA Guidance on Nitrosamine Impurities

The U.S. Food and Drug Administration (USFDA) guideline, "Control of Nitrosamine Impurities in Human

Drugs" (2021), provides recommendations to the pharmaceutical industry regarding the assessment and control of nitrosamine impurities in drug substances and products. This guideline was developed due to the presence of carcinogenic nitrosamine impurities N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in various drug substances and products, including angiotensin II receptor blockers (ARBs), ranitidine, nizatidine, and metformin since 2018. The risk-based approach recommended by the FDA includes risk assessment to identify the potential risk of nitrosamine impurities in drug substances and products. Risk assessment should be performed for the entire drug substance and drug product, including the APIs and excipients. The risk assessment should be focused on the drug substances and products containing amine functional groups that have the potential to be converted to nitrosamines. The detection of impurities should be performed using sensitive and specific detection techniques with low detection limits to detect impurities at levels below the acceptable intake (AI) limits set by the FDA.

The FDA has established conservative AI levels, for example, 18 ng/day, for some nitrosamines. This approach minimises patient risk. Manufacturers must validate these methods pre- and post-approval to ensure product safety. Control strategies involve modifying synthetic pathways, replacing materials, and improving processes to reduce nitrosamine formation, as well as using purge methods to remove nitrosamines from intermediates and final products. Manufacturers must take appropriate actions if nitrosamines exceed AI levels, including recalling products if necessary. (20,25) Risk communication between manufacturers and regulatory agencies is emphasised in the guidance, with an emphasis on transparency in reporting nitrosamine presence or risk mitigation results. This approach protects patient health while supporting pharmaceutical supply. This FDA guidance, published in 2021, provides a framework for risk assessment, analytical detection, control strategies, and communication, all aimed at minimizing patient exposure to nitrosamine-containing human drugs, promoting safety and regulatory compliance.

The FDA has recommended a three-step approach for managing nitrosamine impurities in APIs and drugs. Manufacturers and applicants of APIs and drugs should assess the risk of nitrosamine impurities in APIs, drugs, products under approved applications, and products under pending applications. This assessment should be conducted on the basis of prioritisation. Manufacturers and applicants should maintain these documents and provide them upon request. Once there is an indication that there is a potential risk from nitrosamine, testing should be conducted. Given the low molecular weight, volatility, and high toxicity of nitrosamines, analytical methods should have high specificity, excellent separation, and high sensitivity.

Manufacturers are advised to use strategies that prevent or reduce nitrosamine impurities in APIs and drug products. Any changes that are made to prevent or reduce nitrosamine impurities are to be reported to the FDA as per regulatory requirements by updating regulatory

submissions, such as drug master files (DMFs), NDAs, or ANDAs. The acceptable intake limits of nitrosamine impurities are defined as the “maximum amount of a nitrosamine that can be ingested daily that is deemed safe over a lifetime.” The limits are determined by data from

carcinogenic potency to ensure that there is little or no risk of cancer from long-term exposure. The FDA has established acceptable intake limits for some nitrosamines that are found in drug products. The limits are expressed as ng/day. The acceptable intake limits are as follows:

Table 3. Acceptable intake limits of various nitrosamines as per the US FDA (20)

Nitrosamine	Acceptable Intake Limit (ng/day)
N-Nitrosodimethylamine (NDMA)	96
N-Nitrosodiethylamine (NDEA)	26.5
N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)	96
N-Nitrosodiisopropylamine (NDIPA)	26.5
N-Nitrosoethylisopropylamine (NEIPA)	26.5

These are acceptable limits within which these substances can be consumed. These limits are based on ICH guideline M7 (R1) for the assessment of DNA-reactive impurities in pharmaceuticals. In the event that there are multiple nitrosamines present, the total amount must be within these acceptable daily limits. The FDA demands that these levels be maintained throughout the drug’s lifecycle. Changes to be made to these levels regarding nitrosamine impurities must be notified to the FDA. Such changes must be made through proper documentation. In the event that changes are made to the API drug master files regarding modifications from risk assessment, an amendment must be made. Manufacturers who use these drug master files must be notified. These include DMF, ANDA, and NDA submissions.

8. EMA Regulatory Framework

The European Medicines Agency (EMA) handled the issue of nitrosamine impurities in medicinal products through the Article 5(3) review procedure of Regulation (EC) No. 726/2004. This enabled the Committee for Medicinal Products for Human Use (CHMP) to provide an opinion on nitrosamine impurities in medicinal products. Following the assessment of the issue of nitrosamine impurities in medicinal products, the EMA produced guidance to help marketing authorisation holders implement the recommendations of the Article 5(3) referral procedure. The guidance was presented in a question-and-answer format. It aimed to clarify the process of evaluating, testing, and controlling nitrosamine impurities in active pharmaceutical ingredients and medicinal products. The Q&A document of the EMA on the issue of nitrosamine impurities in medicinal products has undergone revisions based on new scientific knowledge and regulatory considerations.

Updates in 2023 have provided clarifications regarding the control of N-nitrosamines that exceed acceptable intake levels while implementing corrective actions. The updates have also provided the Carcinogenic Potency Categorisation Approach (CPCA) for N-nitrosamines. The CPCA categorises N-nitrosamines according to their predicted carcinogenic potency using toxicological data and structural relationships. The acceptable intake limits of N-nitrosamines according to the ICH M7 guideline will be established using the CPCA if substance-specific carcinogenicity data are unavailable. N-nitrosamines that

have negative test outcomes in the enhanced Ames test using the GLP will be set at an acceptable intake limit of 1.5 µg per day. In contrast, N-nitrosamines that have positive mutagenicity test outcomes will be set at an acceptable intake limit according to their carcinogenic potency.

The importance of lifecycle management of impurities related to the control of nitrosamines is stressed by the EMA. Marketing authorisation holders, manufacturers of APIs, and manufacturers of finished products must manage the risk of the generation of nitrosamine impurities during manufacturing and storage. Risk evaluations must be updated based on the availability of new data. The regulatory authorities of the EU monitor this topic and cooperate internationally to guarantee that medicinal products comply with the requirements of quality, safety, and efficacy while controlling nitrosamine impurities. The European Medicines Agency (EMA) has established provisional acceptable intake (AI) levels of nitrosamine impurities based on the carcinogenicity of the compounds. The AI levels of the compounds are based on the TD50 values of the compounds for the measured nitrosamines or structural analogues using structure-activity relationships. The EMA categorises nitrosamines as "cohort of concern" compounds according to the ICH M7(R1) guideline owing to their mutagenic and carcinogenic properties.

The limits of AI are set conservatively to avoid risks in patients. These limits are subject to revision based on new developments in the mechanisms of action and toxicology. The European Medicines Agency (EMA) uses a tiered approach to determine the acceptable amount of a drug based on the chemical structure of the impurity in the nitrosamine. Some of the typical AI limits set by the EMA include N-nitrosodimethylamine (NDMA), which should not exceed 96 ng/day, and N-nitrosodiethylamine (NDEA), which should not exceed 26.5 ng/day. These limits may be adjusted based on a read-across approach in assigning nitrosamines with similar structures to a potency category with limits of AI. European regulatory agencies require manufacturers of drug substances and products to perform a risk assessment and implement controls to reduce the levels of nitrosamines in the drug substance and product to ensure patient safety. The AI limits for nitrosamines are conservative limits aimed at reducing the risk of carcinogenicity in pharmaceuticals.

Table 4. Acceptable Intake Limits of Various Nitrosamines as per EMA

Nitrosamine Impurity	Acceptable Intake (AI) Limit (ng/day)	Notes
N-Nitrosodimethylamine (NDMA)	~96	Most studied nitrosamine; AI based on rodent carcinogenicity TD50 values
N-Nitrosodiethylamine (NDEA)	~26.5	Adjusted AI limit reflecting higher carcinogenic potency compared to NDMA
N-Nitroso-meglumine (NNM)	1500	Nitrosamine drug substance-related impurity (NDSRI) with comparatively higher AI
Other nitrosamines (various)	Varies (usually < 26.5 - 1500)	AI limits assigned based on structure-activity relationships (SAR) and carcinogenicity

These limits are provisional and based on structure-activity analysis and carcinogenic data to minimise patient risk from probable human carcinogens. Manufacturers use AI values to control nitrosamine levels in drug products.

9. WHO Regulatory Guidelines

The World Health Organization (WHO) deals with the issue of impurities in pharmaceutical products through the WHO Technical Report Series (TRS) and regulatory documents. These guidelines are mainly focused on maintaining the quality, safety, and efficacy of pharmaceutical products in the global pharmaceutical supply chain. WHO guidelines on the issue of impurities in pharmaceutical products highlight the need for strong regulations in the global pharmaceutical supply chain due to the globalisation of pharmaceutical manufacturing. There is a need to avoid contamination and impurities during the manufacturing of active pharmaceutical ingredients (API) and finished pharmaceutical products. WHO guidelines on the issue of impurities in pharmaceutical products promote a risk-based approach by the regulatory authorities and the pharmaceutical industry in dealing with the issue of impurities in pharmaceutical products. It emphasises the evaluation and mitigation of the risk of impurities during the manufacturing process.

WHO recommendations comply with international regulatory requirements, especially the ICH M7 guideline on the assessment of DNA-reactive (mutagenic) impurities in medicinal products. WHO recommendations on the acceptable limits of mutagenic impurities in medicines indicate that these acceptable limits should be established using toxicological assessments and risk-based methodologies. The acceptable intake limits of impurities should be based on carcinogenic risk evaluation to ensure that patients are exposed to a minimum risk during the long-term use of medicinal products. WHO regulatory recommendations on the acceptable limits of mutagenic impurities in medicines play an important role in developing countries because of the existence of different regulatory requirements. WHO recommendations on the acceptable limits of mutagenic impurities in medicines assist countries in developing regulatory requirements. Developing countries are encouraged to use risk-based

control of impurities in medicines based on WHO recommendations.

WHO recommendations promote the harmonisation of regulations and the integrity of medicinal products in the supply chain. The WHO guidelines are in agreement with the main regulatory authorities on the acceptable intake (AI) of nitrosamine impurities by the U.S. Nitrosamines are probable or possible carcinogens and are set at stringent levels to avoid health risks. The nitrosamines are probable or possible carcinogens. These are set at stringent levels to avoid health risk. The acceptable intake levels are set in the range of nanogramme per day (ng/day) to avoid exposure. The acceptable intake levels are set at 18 ng/day for N-nitrosodimethylamine (NDMA), although the actual value may vary depending on the nitrosamine and the exposure route.

WHO and regulatory authorities emphasise risk assessment for nitrosamine impurities in pharmaceuticals, including source, maximum daily dose, treatment duration, patient population, and trace levels. If limits are exceeded for nitrosamine impurities in pharmaceuticals, such batches should not be marketed. Emphasis is given to control measures such as optimization processes, reformulation strategies, selection of alternative materials, and analytical techniques such as LC-MS/MS and GC-MS/MS to detect impurities at acceptable levels. Interaction between pharmaceutical companies and regulatory authorities ensures that these limits are scientifically justified to ensure public health. WHO has set very low levels of acceptable intake limits for nitrosamine impurities to ensure minimum risk of carcinogenesis.

Acceptable intake limits vary. USFDA uses generally 26.5 ng/day. EMA uses compound-specific limits in Appendix 1. The class limit for the compound is 18 ng/day. WHO endorses shared interim values. The classification also differs. The USFDA uses NDSRIs and structure-activity categories. The EMA uses the CPCA and enhanced Ames test. The WHO emphasises probable carcinogens. Risk management also differs. The USFDA uses a three-step mitigation. The EMA requires portfolio reviews. WHO emphasizes cross-contamination.

Table 5. Interim allowable daily intake limits for N-nitrosamine impurities as per the WHO

Impurity Abbreviation	Chemical Name	Allowable Daily Intake (AI)
NDMA	N-Nitrosodimethylamine	96.0 ng/day
NDEA	N-Nitrosodiethylamine	26.5 ng/day
NMBA	N-Nitroso-N-methyl-4-aminobutyric acid	96.0 ng/day

DIPNA	N-Nitrosodiisopropylamine	26.5 ng/day
EIPNA	N-Nitrosoethylisopropylamine	26.5 ng/day

10. Comparative Regulatory Analysis

Table 6. Comparison of Regulatory Requirements

Parameter	USFDA	EMA	WHO
Risk assessment	Three-step strategy: assess APIs/drug products, test if risk identified, and implement controls. Prioritise by dose/duration.	Stepwise approach for all chemically synthesised APIs and lifecycle management, including changes.	Comprehensive assessment of sources, such as premises and equipment, and a broad scope beyond synthesis.
Acceptable intake approach	Compound-specific AIs (e.g., NDMA, 96 ng/day; NDEA, 26.5 ng/day); default, 26.5 ng/day; potency categories for NDSRIs; TTC, if needed.	ICH M7-based AIs: Appendix 1 lists (e.g. class-specific 18 ng/day) CPCA/EAT for unknowns.	Adopts interim AIs like regulators (e.g., NDMA/NDEA); ICH M7 for others; maintains as low as possible.
Analytical methods	Sensitive LC-HRMS, LC-MS/MS, and GC-MS/MS; validated for low ppb levels; FDA methods published.	Highly sensitive validated methods, updates for non-mutagenic nitrosamines, sensitivity requirements in Q&A 9.	Appropriate sensitive methods; aligns with global methods (e.g., LC-MS/GC-MS); test to confirm below limits.
Reporting requirements	Report changes per 21 CFR 314.70/97; timelines: complete assessment/testing by October 2023 (marketed), submit mitigations.	Notify authorities regardless of level; templates; deadlines passed (for example, September 2022 chem synth, July 2023 biologics).	Verify levels and request MAHs to assess/control; interim limits during transitions.

11. Control Strategies for Nitrosamine Impurities

Control strategies for the management of nitrosamine impurities in pharmaceuticals include risk management, process optimisation, raw material management, and quality by design principles to minimise the risk of patient exposure to these probable human carcinogens.

- Risk Assessment:** The risk evaluation aspect is centred on the manufacturing process. Major aspects include the evaluation of the synthesis route of the active pharmaceutical ingredient (API) and the evaluation of excipients for the presence of nitrosamines. This includes the evaluation of the presence of nitrosating agents and amines that may result in the formation of nitrosamines. This helps in the prioritisation of materials to be monitored.
- Process optimisation:** Once the risk has been identified, modifications to the processes can help prevent the formation of nitrosamines. This involves altering processes to prevent the formation of nitrosating agents and changing the routes to prevent the formation of nitrosamines. Additionally, parameters such as pH and temperature are optimised to prevent the formation or facilitate the removal of nitrosamines. Reformulations or reagents that prevent the formation of nitrosamines are used.
- Raw Material Control:** Control starts with supplier qualification and raw material testing to exclude nitrosamine precursors or impurities. For raw materials, the supplier must demonstrate controls in place, and raw material testing may be conducted for nitrosamine contamination where applicable. This helps reduce variability and unexpected nitrosamine formation outside of manufacturing.
- Quality by Design (QbD):** In implementing risk-based impurity control as part of QbD, there is a need

to design and control processes in the pharmaceutical industry. QbD is a method that helps identify parameters affecting nitrosamine formation. In-process controls, including LC-MS/MS and GC-MS/MS, are used for nitrosamine detection.

Therefore, nitrosamine impurity control strategies must be developed by considering risk assessment, process optimisation, raw materials, and the quality by design concepts. These strategies are consistent with those proposed by the FDA, EMA, WHO, Health Canada, and other regulatory bodies to ensure the safety of pharmaceutical products.

12. Future Perspectives

Future perspectives for nitrosamine-related impurities in pharmaceuticals are centred around advancing predictive tools, global guidelines, and analytical techniques. The use of artificial intelligence for impurity prediction is a new paradigm that has shown promise. Computational models, such as structure-activity relationships (SAR), quantitative structure-activity relationships (QSAR), and machine learning algorithms, are effective tools for predicting the potential of nitrosamine-related impurities to form carcinogenic byproducts, as well as novel nitrosamine drug substance-related impurities (NDSRIs), without prior empirical data. These models are effective in identifying potential hazards and classifying nitrosamines according to their metabolic activation potential. However, challenges are encountered during the prediction of nitrosamine-related impurities that are structurally diverse; thus, it is important to use in vivo data and expert assessment as a complement to prediction models. Global guidelines are important for ensuring consistency in risk assessment, control strategies, and compliance across different countries. Regional regulatory differences require a dialogue between regulatory authorities to ensure

that a consensus is reached on acceptable intake thresholds, testing requirements, and controls.

Recent collaborations between the FDA, EMA, Health Canada, and WHO are working to standardize methodologies and frameworks. These are helping to speed the approval of products and the safe operation of the pharmaceutical supply chain. Improved analytical technologies play a critical role in the detection of nitrosamines at ultra-trace levels. Techniques such as ultra-sensitive liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), high-resolution mass spectrometry (LC/HRMS), and ion mobility spectrometry are improving the sensitivity, specificity, and speed of nitrosamine impurity profile characterisation. These technological advancements allow the simultaneous quantification of multiple nitrosamine impurities in complex pharmaceutical matrices with high accuracy and low levels of quantification. Validation of the analytical method in accordance with ICH guidelines guarantees the effective application of the technology. The development of simplified and fast analytical techniques will be crucial for the real-time monitoring and risk mitigation of the manufacturing process. Future directions in the development of nitrosamine impurity control technology, including the incorporation of artificial intelligence (AI) prediction technology and the standardisation of methodologies by international regulatory bodies, hold promise for enhancing the safe production of pharmaceutical products.

13. Conclusion

Nitrosamine impurities are a critical regulatory concern from a safety perspective owing to their genotoxic and carcinogenic risk in the pharmaceutical industry. Risk management strategies for nitrosamine impurities involve synthetic routes, excipient sources, and manufacturing processes to manage risk. These strategies allow for the implementation of measures to improve safety while ensuring regulatory compliance. Global harmonisation is critical to ensure consistent standards, limits, and guidelines across regions. Harmonisation will allow efficient risk management strategies to be implemented, thereby easing regulatory burdens and ensuring patient safety. Risk management strategies for nitrosamine impurities include assessment and international regulatory frameworks.

Acknowledgements

I would like to express my gratitude to my Mentor/, who help me in writing & reviewing in this article. I would like to express my gratitude to International Journal of Drug Regulatory Affairs who gave me the opportunity to publish the article.

Financial Disclosure statement:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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