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Review Article

Nitrosamine Impurities in Pharmaceutical Products: A Comprehensive Review of Sources, Analytical Detection Methods, Toxicological Risks, and Regulatory Guidelines

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ABSTRACT

Nitrosamine impurities pose a significant health risk in pharmaceutical products due to their carcinogenic, mutagenic, and DNA-damaging properties. These organic compounds can form from various sources such as raw materials, intermediates, solvents, and packaging materials used in the manufacturing and storage of active pharmaceutical ingredients (APIs) and finished drug products. Contamination has been identified in drugs like ranitidine, nizatidine, metformin, and certain angiotensin II receptor blockers (ARBs). This review provides a comprehensive overview of the chemistry, sources, formation mechanisms, and environmental influences contributing to nitrosamine formation. It also discusses key analytical techniques for detection and quantification across APIs, excipients, and packaging components. Additionally, current global regulatory frameworks from the EMA, FDA, and USP are summarized, offering insights into permissible limits and compliance strategies. The article further explores challenges and preventive measures to control nitrosamine contamination, guiding pharmaceutical industries in quality assurance and risk management practices.

Keywords: Nitrosamine Impurities, Pharmaceutical Contamination, Detection Techniques, Health Risks, Regulatory Guidelines.

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1. **Introduction**

Nitrosamines are groups of organic compounds that contain nitroso group (N=O) linked to an amine group (NH2). Nitrosamines are widely distributed in the environment and can be found in various sources such as water, food, tobacco, pesticides, or plastics [1]. Nitrosamines are also generated endogenously in the human body through the interaction of nitrites and amines [2].

Nitrosamines are of great concern for public health because they are potent carcinogens that can cause tumours in various organs like the liver, kidney, bladder, stomach, oesophagus, and pancreas [2]. The International Agency for Research on Cancer (IARC) has classified some nitrosamines as human carcinogens (Group 1) and others as probable human carcinogens (Group 2A) [3].

Nitrosamine impurities in pharmaceutical products have become a critical issue for global health authorities and pharmaceutical manufacturers. In 2018, the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) detected the presence of N-nitroso dimethylamine (NDMA) and N-nitroso diethylamine (NDEA), which are the two most common and carcinogenic nitrosamines, in several batches of angiotensin II receptor blockers (ARBs), a class of drugs used to treat hypertension and heart failure [4,5]. Since then, several other nitrosamine impurities identified in other drug products, such as ranitidine, metformin, nizatidine, propranolol, varenicline and rifampicin [6,7]. In a recent publication, besides detailed discussions on the chemistry of nitrosamine formation, the results of a computational screening of more than 12,000 small molecules from the GSRS database to assess their nitrosamine potential. They reported that many of these compounds contain secondary or tertiary amines that can undergo nitrosation, resulting in many possible nitrosamine contaminants. Specifically, they found that 40.4 % of the active pharmaceutical ingredients (APIs) and 29.6 % of the API impurities had this risk [7,8].

Nitrosamines are the class of mutagenic impurities that are formed by the reaction of nitrites with secondary or tertiary amines. Nitrosamines, especially the small dialkyl type, have high genotoxic potential and are categorized as an ICH M7 cohort of concern[9]. Consequently, they are not subject to the defaulting threshold of toxicological concern (TTC) of 1.5 µg/day [10]. In the absence of substance-specific limits obtained from carcinogenicity tests or read-across methods using close structural analogs[11], the defaulting acceptable intake was established by the European Medicines Agency (EMA) at 18 ng/day, based on the 5th percentile of TD50 values for nitroso compounds from Lhasa Carcinogenicity Database (LCDB)[12] and a linear extrapolation to theoretical cancer risk of 10-5. The Food and Drug Administration (FDA) adopted the same limit of 26.5 ng/day for N-nitroso diethylamine (NDEA), a representative nitrosamine and for N-nitroso dimethylamine (NDMA), another nitrosamine impurity, at 96 ng/day. This limit is based on a theoretical additional cancer risk of 1 in 100,000 over the lifetime of exposure.

A revised version of the Q&A document on nitrosamine impurities in human medicinal products was released by EMA on July 7th, 2023. This version introduces a new SAR-based method for AI determination of nitrosamines, using five potency categories based on the carcinogenicity data of known nitrosamines. The default AI for potency category 1 is 18 ng/day, while the AIs for categories 2, 3, 4 and 5 are 100 ng/day, 400 ng/day, 1500 ng/day and again 1500 ng/day, respectively [9].

Nitrosamine impurities are a global concern for the quality and safety of pharmaceutical products, and they are classified as probable human carcinogens by the International Agency for Research on Cancer (IARC). To detect and quantify these impurities, different analytical methods have been developed using LC or GC chromatography, headspace or split/spitless injection, L 43, G-16, or L 1 columns/phases, and HRMS or MS-MS with triple quadrupole detection. The methods comply with the regulatory standards for nitrosamine analysis in pharmaceutical samples. The EMA and the FDA have provided guidance and recommendations for the testing, reporting, and mitigation of nitrosamine contamination in APIs and FDPs [10,11].

This review article intends to provide a comprehensive overview of the significance of nitrosamine impurities in pharmaceutical drug products, their potential health risks, and the necessity for stringent regulatory standards. The article will also discuss the possible sources and mechanisms of nitrosamine formation, the analytical methods for detection and quantification, and the strategies for the prevention and control of these impurities in the pharmaceutical industry.

2. FDA, USP and EP posted testing methods

Regulatory agencies like the U.S. Food and Drug Administration (FDA), United States Pharmacopeia (USP), and European Pharmacopoeia (EP) have developed and published specific analytical methods for the detection of nitrosamine impurities in pharmaceutical products. These methods target a variety of compounds and are tailored to different drug classes such as ARBs, ranitidine, metformin, and antibiotics like rifampin. Each regulatory body provides validated techniques including gas chromatography (GC), liquid chromatography (LC), and mass spectrometry-based methods to ensure accurate and sensitive detection. The availability of reference standards and harmonized testing strategies plays a critical role in supporting compliance and maintaining drug safety (Table 1).

Table 1: Comparison of Testing Methods for Nitrosamine Impurities as Posted by FDA, USP, and EP Across Selected Drug Categories

Category	ARBs	Ranitidine	Metformin	Rifampin and Rifapentine	USP Chapter <1469>	European Pharmacopoeia (EP) General Chapter 2.5.42
Title	FDA, USP and EP Posted Testing Methods	-	-	-	Nitrosamine Impurities	Nitrosamines in active substances
Methods	6 methods (GC-MS HS, GC-MS DI, LC-	2 methods (LC-HRMS)	2 methods (LC-HRMS)	1 shared method (LC-HRMS)	4 methods (LC-HRMS,	3 methods (GC-LC HRMs, Lc-

	HRMS, Rapid Fire)				Lc-MS/MS, Gc-MS/MS)	Ms/Ms, Gc-Ms/Ms)
Compounds	NDMA, NDEA, NMBA, NDBA, NDIPA, NEIPA	NDMA	NDMA, NDEA, NMBA, NEIPA, NDIPA, NDPA, NMPA, NDBA	MNP, CNPP	6 USP reference standards are available	7 reference standards are available (NDMA, NDEA, NMBA, NDIPA, NDPA, NEIPA, NDBA)

Chemical Structure, Formation Mechanisms, and Occurrence in Various Matrices

Nitrosamines are categorized by the presence of a nitroso group (N=O) bonded to an amine group (NH2). The general formula of nitrosamines is R1N(-R2)-N=O, where R1 and R2 can be alkyl or aryl groups [12] (Table 2. Figure 1).

The nitrosamine is a set of carcinogens generated through the reactions by nitrite or other nitrogenous nitrite secondary and tertiary amines, amides, carbamates, and urea derivatives. The nitrosamine impurities can arise through the nitrosating reaction from acidic nitrate salts like secondary, tertiary, or quaternary amines to nitrous acid-like acidic salts [13].

Nitrosamines are classified into two main types: volatile and non-volatile. Volatile nitrosamines are those that have low molecular weight and high vapour pressure, such as NDMA and NDEA. Non-volatile nitrosamines are those that have a high molecular weight and a low vapor pressure, such as N-nitrosopiperidine (NPIP) and N-nitrosodiisopropylamine (NDIPA)[17]. These impurities have diverse physicochemical properties that affect their formation, detection, and removal as shown in Table 1. Most nitrosamine impurities are polar compounds, but they differ [17] in their ionization behaviour. The majority are neutral molecules, such as NDMA, NDEA, NEIPA, NDPA, NDIPA, NDBA, NMPA, NMOR, and NPIP. Some are basic compounds, such as MNP and CPNP, and one is an acidic compound, NMBA[18]. The solubility of nitrosamine impurities also varies depending on their structure. NDMA and NDEA are highly soluble in water, while NDPA and NDBA have lower water solubility. Common organic solvents used for extraction and analysis of nitrosamine impurities include dichloromethane, methanol, and acetone. Nitrosamine impurities might be degraded at high temperatures, resulting in loss of detection or formation of secondary amines. Therefore, it is important to evaluate the physico-chemical properties of active pharmaceutical ingredients (APIs) and other components of drug products that may be sources or precursors of nitrosamine impurities. For example, APIs that can degrade to form secondary amines may increase the risk of nitrosamine formation.

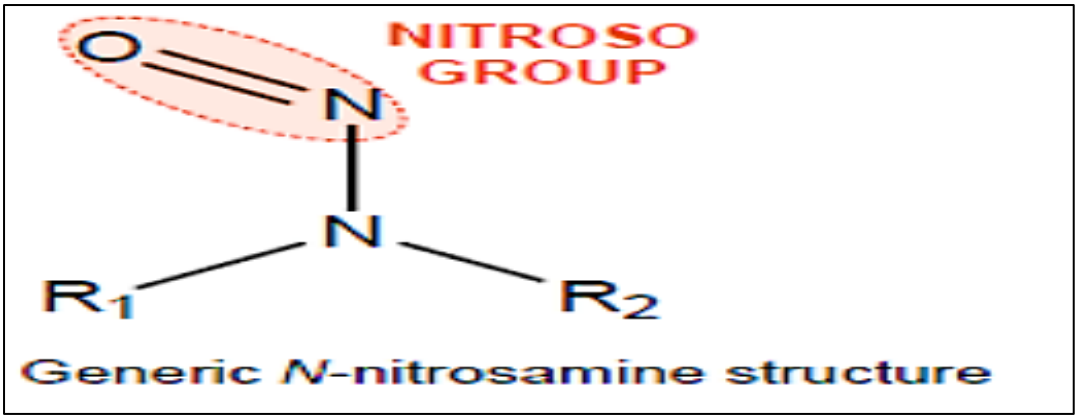
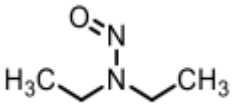
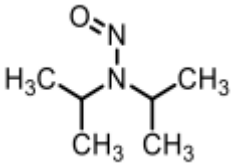
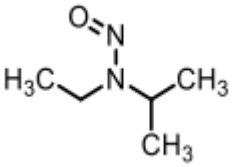
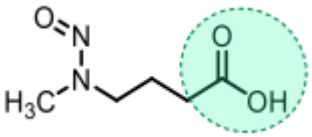
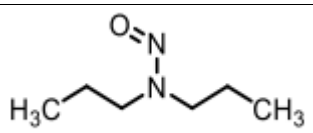
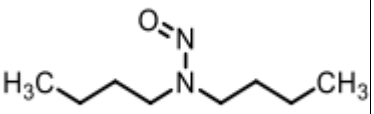
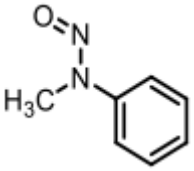
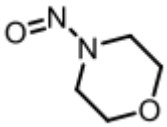
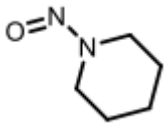
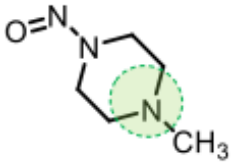
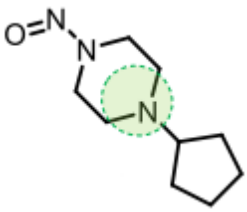


Figure 1: General structure of nitrosamine

Table 2: List of Nitrosamine impurities and their physicochemical properties [14–16]

Sr.No.	Name of Impurity	Structure	Molecular Formula	Molecular Weight	Boiling Point
1	N-Nitrosodimethylamine (NDMA)		C2H6N2O	74.08 g/mol	152 °C
2	N-Nitrosodiethylamine (NDEA)		C4H10N2O	102.14 g/mol	174 °C
3	N-Nitrosodiisopropylamine (NDIPA)		C6H14N2O	130.19 g/mol	215 °C
4	N-Nitrosoethylisopropylamine (NEIPA)		C5H12N2O	116.16 g/mol	193 °C
5	N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)		C5H10N2O2	130.15 g/mol	208 °C

6	N-Nitrosodipropylamine (NDPA)		C6H14N2O	130.19 g/mol	230 °C
7	N-Nitrosodibutylamine (NDBA)		C8H18N2O	158.25 g/mol	267 °C
8	N-Nitrosomethylphenylamine (NMPA)		C7H8N2O	136.15 g/mol	247 °C
9	N-Nitrosomorpholine (NMOR)		C4H8N2O2	116.12 g/mol	178 °C
10	N-nitrosopiperidine (NPIP)		C5H10N2O	114.15 g/mol	163 °C
11	N-Nitrosomethylethylamine (MNP)		C3H8N2O	88.11 g/mol	145 °C
12	N-Nitrosocyclopentylmethylamine (CPNP)		C6H12N2O	128.17 g/mol	200 °C

Nitrosamine impurities can be formed through various mechanisms, depending on the source and the conditions. The most common mechanism is the nitrosation reaction, which involves the transfer of a nitroso group from a nitro-sating agent to an amine group [10,21]. Nitro sating [22,23]. Amines can be primary, secondary, or tertiary, and may be derived from natural or synthetic sources, such as amino acids, proteins, drugs, or pesticides [10]. The nitrosation reaction can be influenced by several factors, such as pH, temperature, oxygen, light, metal ions, and catalysts. Other possible mechanisms for nitrosamine formation include the degradation of nitroso derivatives, the oxidation of secondary amines, the reduction of nitro compounds, the

rearrangement of N-nitroso compounds, and the transmigration of amines by other nitrosamines.

The formation of nitrosamine impurities in pharmaceutical products or substances may be a serious quality and safety issue, as nitrosamines are probable human carcinogens. Therefore, it is important to identify the probable sources and precursors of nitrosamine formation and to implement appropriate control strategies to prevent or minimize their occurrence. Some of the possible sources and precursors of nitrosamine formation are the API or its impurities, the excipients or their impurities, the manufacturing process or equipment, and storage or transportation situations. Some of the possible control strategies to prevent or minimize nitrosamine formation are the selection of appropriate API and excipient sources, the optimization of the manufacturing process and equipment, the establishment of suitable storage and transportation conditions, and the implementation of risk assessment and mitigation plans. The application of quality by design and quality risk management principles can help to identify and control the critical quality attributes and critical process parameters for nitrosamine impurities or their precursors [17].

Nitrosamine impurities can occur in various matrices, such as water, food, tobacco, cosmetics, and pharmaceutical products. In water, nitrosamines can be formed by the reaction of chlorinated disinfection by-products, such as chloramines, with organic nitrogen compounds, such as urea, ammonia, or amines. This can happen in drinking water, wastewater, or industrial water, where chlorination is used to control microbial growth or organic matter. For example, NDMA, a common nitrosamine impurity, can be formed by the reaction of chloramine with dimethylamine, which can be derived from natural sources, such as algae, or synthetic sources, like detergents or pesticides [18,19].

These chemical compounds can be formed in food, tobacco, or cosmetics by the reaction of nitrites with amines. Nitrites are often used as preservatives in food, but they can also be found naturally in tobacco leaves. Amines are organic molecules that can be present in meat, fish, cheese, vegetables, nicotine, alkaloids, surfactants, emulsifiers, or fragrances. When nitrites and amines come into contact, especially under high temperatures or acidic conditions, they can form nitrosamines, which are known to be carcinogenic. Some examples of nitrosamine impurities are NDEA, which may be formed in food by the reaction between nitrite with diethylamine; NNK, which may be formed in tobacco by the reaction between nitrite with nicotine; and NDELA, which can be formed in cosmetics by the reaction of nitrosyl chloride, a nitrosating agent, with diethanolamine [19–21].

In pharmaceutical products, nitrosamines can be formed by the reaction of nitrosating agents, which can be presented as reagents, catalysts, solvents, or contaminants, with amines, which can be present in drug substances, excipients, or packaging materials. This can happen during the synthesis, purification, formulation, or storage of pharmaceutical products, where nitro-sating agents can be derived from nitrite, nitrate, nitro compounds, or other sources, and amines can be derived from drug substances, like sartans, ranitidine, or metformin, or excipients, such as sodium nitrite, sodium starch glycolate, or crospovidone. For example, NDMA, NDEA, NMBA, and other nitrosamine impurities have been detected in several sartan products, which are angiotensin II receptor blockers used to treat

hypertension and heart failure. These impurities can be formed by the reaction of nitrite or nitrosamine reagents with the tetrazole or diazole ring of the sartan drug substances, such as valsartan, losartan, or irbesartan [22–24].

Nitrosamine Impurities in Different Classes of Drugs

Nitrosamine impurities have been detected in different classes of drugs, such as sartans, ranitidine, nizatidine, and metformin. The formation of these impurities within specific drug classes can be attributed to different reasons, such as the chemical structure of the drug substance, the manufacturing process, the storage conditions, or degradation pathways [25].

Sartans are a class of drugs that act as angiotensin II receptor blockers (ARBs) and are used to treat hypertension and heart failure. Sartans have a tetrazole ring in their structure, which can act as a nitrosating agent under acidic conditions. Sartans can also contain amines or amides in their structure, which can act as nitrosatable groups. Therefore, sartans can undergo intramolecular or intermolecular nitrosation reactions, resulting in the formation of nitrosamine impurities, such as NDMA, NDEA, N-nitroso-N-methyl-4-aminobutyric acid (NMBA), NDIPA, or N-nitrosoethylisopropylamine (NEIPA) [26]. Ranitidine is a drug that acts as a histamine H2-receptor antagonist and is used to treat peptic ulcers and gastroesophageal reflux disease. Ranitidine has a nitro group and a dimethylamine group in its structure, which can react with each other under acidic conditions, forming NDMA as a by-product. Ranitidine can also react with nitrite, which can be present in the stomach or the environment, forming NDMA as a by-product. Ranitidine can also degrade to NDMA under high temperatures or prolonged storage [27]. Nizatidine is a drug that acts as a histamine H2-receptor antagonist and is used to treat peptic ulcers and gastroesophageal reflux disease. Nizatidine has a nitro group and a dimethylamine group in its structure, like ranitidine, and can form NDMA through the same mechanisms as ranitidine [27]. Metformin is a drug that acts as a biguanide and is used to treat type 2 diabetes mellitus. Metformin has a guanidine group in its structure, which can act as a nitrosatable group. Metformin can react with nitrosating agents, such as nitrite, nitrate, or nitric oxide, forming NDMA as a by-product. Metformin can also degrade to NDMA under high temperatures or prolonged storage [28] (Table 3).

Table 3: List of nitrosamine impurities in different classes of drugs

Drug Class	Nitrosamine Impurity	Mechanism of Formation	References
Sartans	NDMA, NDEA, NMBA, NDIPA, NEIPA	Tetrazole ring acts as a nitrosating agent under acidic conditions; amines or amides in the structure contribute to nitrosation reactions.	[29–32]
Ranitidine	NDMA	Interaction between the nitro group and dimethylamine group; reaction with nitrite or degradation under high temperatures.	
Nizatidine	NDMA	Similar mechanisms as ranitidine due to structural similarities.	

Metformin	NDMA	Formation via nitrosation reactions involving amine groups in the drug substance.	
Valsartan	NDMA, NDEA	Detected in Valsartan-containing products; likely due to tetrazole ring and amine interactions.	
Losartan	NDMA	Potential nitrosation reactions involving the tetrazole ring.	
Irbesartan	NDMA	Similar mechanisms as other sartans.	

Sensitivity requirements for analytical methods

The sensitivity of the analytical method is the ability of the method to distinguish between different concentrations of an analyte. One way to measure the sensitivity is to use the limit of quantification (LOQ), which is the lowest concentration of an analyte that can be quantified with acceptable accuracy and precision. According to the ICH Q2 (R1) guideline, the LOQ is preferred over the limit of detection (LOD) for impurity testing and decision-making. The LOD is the lowest concentration of an analyte that can be detected but not necessarily quantified as an exact value. The LOD is not recommended for setting limits or for assessing the risk of nitrosamines, which may not be completely avoidable in some cases.

An example of applying the LOQ for impurity testing is the case of NDMA in Valsartan, a drug used to treat high blood pressure. The acceptable intake (AI) of NDMA, based on the toxicological risk, is 0.3 ppm (0.0003 mg/mL) or 96 ng/day for a 320 mg/day dose of Valsartan. Therefore, the analytical method used to measure the NDMA level in Valsartan should have an LOQ that is equal to or lower than the acceptable intake (AI) and should also meet the regulatory recommendations for sensitivity. Different regulatory agencies have issued guidelines for the control of nitrosamine impurities in human drugs, which specify the required sensitivity of the analytical methods. the analytical methods should have a LOQ that is equal to or lower than 0.03 ppm for products with a maximum daily dose (MDD) of less than 880 mg/day. For products with a MDD of more than 880 mg/day, the LOQ should be as low as reasonably practical. The test results should be lower than the acceptable intake (AI) of the respective nitrosamine impurity, which is based on the toxicological risk.

According to the EMA assessment report [33], the analytical methods should have a LOQ that is equal to or lower than the acceptable limit (AL) for the respective nitrosamine impurity, considering the purpose of testing. For routine control, the LOQ should be equal to or lower than AL. For justifying skip testing, the LOQ should be equal to or lower than 30% of the AL. For justifying omission from the specification, the LOQ should be equal to or lower than 10% of the AL. Exceptions may be needed depending on the MDD or if more than one nitrosamine is expected to be present. This belonging should be discussed with the relevant competent authorities.

Analytical Methods for Detecting Nitrosamines

Nitrosamine impurities are a class of mutagenic compounds that are probable human carcinogens. They may be present in a few active pharmaceutical ingredients (APIs) and drug products (DPs) at low levels, which pose challenges for testing and detection. Several regulatory agencies and organizations have published testing methods for nitrosamines in different APIs and DPs, using

various analytical techniques. These methods are summarized below:

US FDA: The US Food and Drug Administration (FDA) has published testing methods for nitrosamines in sartans, metformin, and ranitidine, using liquid chromatography (LC) with electrospray ionization high-resolution mass spectrometry (ESI HRMS), gas chromatography (GC) with tandem mass spectrometry (MS), or LC with atmospheric pressure chemical ionization triple quadrupole mass spectrometry (APCI QqQ).

Health Canada/EMA: Health Canada and the European Medicines Agency (EMA) have published testing methods for nitrosamines in sartans and ranitidine, using GC with MS/MS, LC with APCI QTrap, or LC with APCI QqQ [11,42].

USP: The United States Pharmacopeia (USP) has published a general chapter <1469> for nitrosamine impurities, which guides risk assessment, control strategy, limits, testing, and analytical procedures. The chapter also includes testing methods for nitrosamines in sartans, metformin, and ranitidine, using LC with ESI HRMS, GC with MS/MS, or LC with APCI QqQ [29].

Sartan and ranitidine-based drugs have contained nitrosamine impurities, which are potential carcinogens, at unacceptable levels. Several regulators have published testing methods for the detection and quantification of nitrosamine impurities in these drugs, using various analytical techniques. These testing methods are summarized below:

U.S. FDA: The U.S. Food and Drug Administration (FDA) has published testing methods for nitrosamine impurities in sartan-based drugs and ranitidine-based drugs, using liquid chromatography (LC) with electrospray ionization high-resolution mass spectrometry (ESI HRMS), gas chromatography (GC) with tandem mass spectrometry (MS/MS), or LC with atmospheric pressure chemical ionization triple quadrupole mass spectrometry (APCI QqQ).

Health Canada: Health Canada has published a testing method for nitrosamine impurities in sartan-based drugs 4, using GC with MS/MS, LC with APCI QTrap, or LC with APCI QqQ.

Taiwan FDA: The Taiwan Food and Drug Administration (TFDA) has published a testing method for nitrosamine impurities in sartan-based drugs 43, using LC with ESI HRMS or GC with MS/MS.

Council of Europe: The Council of Europe has published testing methods for nitrosamine impurities in sartan-based drugs and ranitidine-based drugs, using GC with MS/MS, LC with APCI QTrap, or LC with APCI QqQ[44].

Health Science Authority Singapore: The Health Science Authority (HSA) of Singapore has published a testing method for nitrosamine impurities in ranitidine-based drugs, using LC with ESI HRMS.

Nitrosamines are a class of carcinogenic compounds that have the N-NO group as a chromophore. They have low molar absorptivity with two maxima of absorption at 230 and 330 nm. Therefore, HPLC-UV be may not suitable to control nitrosamines at or below the established acceptable limits. Several alternative methods have been proposed for the analysis of nitrosamines in pharmaceuticals, such as GC-MS, LC-MS, GC-TEA, and LC-FLD with pre-column fluorescence labelling. GC-MS may increase sensitivity and selectivity for volatile or semi-volatile nitrosamines, but it may also cause thermal degradation of thermally labile APIs (e.g., ranitidine) and form nitrosamine artefacts in the presence of nitrite and acid. LC-MS may also

increase sensitivity and selectivity for an extensive range of nitrosamines, even those not so volatile, but it may require lower temperature in Atmospheric pressure chemical ionization (APCI) to avoid thermal degradation of nitrosamines. GC-TEA has great sensitivity for compounds with the N-nitroso group, but it lacks selectivity for organic nitrites, N-nitroso, C-nitroso, nitrates, and inorganic nitrite. LC-FLD with pre-column fluorescence labelling uses derivatization protocols and analysis by HPLC-FLD to enhance the detection of nitrosamines. These methods have different advantages and limitations, and the choice of the most suitable method depends on the specific application and matrix of interest.

Performance Characteristics of Analytical Methods

The validation of analytical procedures for the detection and quantification of N-nitrosamine impurities in drug products (DPs) and active pharmaceutical ingredients (APIs) requires the evaluation of several performance characteristics, like sensitivity, selectivity, accuracy, precision, linearity, and robustness. The in-depth analysis of performance characteristics of various analytical methods offers a nuanced understanding of their capabilities. Comparative assessments aid in identifying the most suitable techniques for different contexts, such as the type of API/DP, the level of impurity, the purpose of testing, and the regulatory requirements. Several regulators have published testing methods for nitrosamine impurities in different APIs and DPs, using various analytical techniques. The validation of analytical procedures for the detection and quantification of N-nitrosamine impurities in drug products (DPs) and active pharmaceutical ingredients (APIs) requires evaluation of several performance characteristics, such as [34]:

Sensitivity: The sensitivity of the analytical procedure is its ability to distinguish between different concentrations of an analyte. It should be sensitive enough to detect and quantify N-nitrosamine impurities at ppm levels 10, 47, The sensitivity can be measured by the limit of detection (LOD) and the limit of quantification (LOQ), which are the lowest concentrations of an analyte that can be detected and quantified, respectively, with acceptable accuracy and precision.

Selection: The selection of the analytical procedures is the ability to assess explicitly the analyte in the presence of components that may be expected to be present such as impurities, degradants, matrix etc. The procedure should be selective for the target N-nitrosamine impurities.

Accuracy and Precision: The accuracy of an analytical procedure is the closeness of agreement between the test results and the true value of the analyte. The precision of an analytical procedure is the closeness of agreement between a series of measurements obtained from multiple samples of the same homogeneous sample. The accuracy and precision of the procedure should be within the acceptance criteria for the intended application.

Linearity: The linearity of the analytical procedure is its ability to obtain test results that are proportional to the concentration of the analyte within a given range. The procedure should be linear within the proposed concentration range.

Robustness: The robustness of the analytical procedure measures the capacity to remain genuine by small but deliberate variations of the method parameters and provides an indication of its reliability during normal usage.

Analytical methods such as GC/MS, LC/MS/MS, and LC/Q-TOF have been widely applied to detect and quantify trace amounts of nitrosamines in

pharmaceuticals. An inter-laboratory study demonstrated that accurate and precise quantitation of trace-level nitrosamines can be achieved across multiple analytical procedures.

Health Risks Associated with Nitrosamine Exposure

Exposure to nitrosamines has been associated with various adverse health effects, especially their carcinogenic potential. In this article, we review the current evidence on the health risks of nitrosamine exposure, focusing on its mutagenic, carcinogenic, and genotoxic properties. We also discuss the sources, mechanisms, and factors that influence nitrosamine formation and bioactivation, as well as the methods for detection, quantification, and mitigation of nitrosamine impurities in different matrices. We aim to provide a comprehensive and critical overview of the health implications of nitrosamine exposure and to identify the knowledge gaps and research needs for future studies.

The section discusses the potential health risks stemming from nitrosamine exposure, encompassing mutagenicity, carcinogenicity, and genotoxicity. By synthesizing available evidence, the article contributes to a comprehensive understanding of the health implications.

Nitrosamines are organic compounds that have been linked to various health risks, particularly their carcinogenic effects. Studies have shown that nitrosamines are potent carcinogens, meaning they have the potential to cause cancer. Additionally, nitrosamine exposure has been associated with other health conditions, including liver and kidney damage, respiratory issues, and even developmental disorders in infants.

Sources, Mechanisms, and Factors Influencing Nitrosamine Formation

Nitrosamine is generated in various ways like food, beverages, and industrial processes. For example, nitrosamine is formed when certain food ingredients, such as nitrates, react with amino acids or amines under certain conditions. More sources of nitrites include meats, bacon and hot dogs, fish and dairy products.

Detection, Quantification, and Mitigation of Nitrosamine Impurities

There are several methods for detecting, quantifying, and mitigating nitrosamine impurities. To minimize the formation of nitrosamines, regulatory measures have been put in place. Nitrosamine exposure has significant health risks due to its carcinogenic properties. There it is very crucial to understand the sources and their mechanism of nitrosamine formation and to implement useful methods for their detection, quantification, and mitigation.

Acceptable intake limits for Nitrosamines

The US FDA has been actively managing nitrosamine impurities in drug products since 2018, with a focus on N-nitroso dimethylamine (NDMA) observed in valsartan. The Nitrosamine Guidance issued by the FDA in 2020 recommends a three-step process for manufacturers to mitigate nitrosamine impurities: risk assessments, confirmatory testing, and reporting of changes to prevent or reduce nitrosamine presence. Nitrosamines, being potential genotoxic agents and possible human carcinogens, are included in the cohort of concern compounds in the ICH M7(R2) guidance. An Acceptable Intake (AI) limit is defined to approximate a 1:100,000 cancer risk after 70 years of exposure. As the understanding of N-nitroso dimethylamine-related substances (NDRSIs) evolves, the FDA recommends a risk-based safety assessment of NDSRIs, using structure-activity relationship (SAR)

concepts to assess and classify their mutagenic and carcinogenic risk. This approach acknowledges that not all NDSRIs have the same carcinogenic potency, imitated by different recommended AI limits for each potency group.

Establishing an Acceptable Intake (AI) limit for N-nitroso dimethylamine-related substances (NDSRIs) is complex due to their uniqueness to each Active Pharmaceutical Ingredient (API) and the often limited or non-existent safety data. The FDA has communicated AI limits for a few NDSRIs, but most remain undetermined. AI limits are based on safety assessments, including the evaluation of mutagenic and carcinogenic potential. These limits represent levels at which the FDA has determined that impurities would not pose a safety concern for patients. AI can be calculated using rodent carcinogenic potency data, such as TD50 values, available from databases like the Carcinogenic Potency Database (CPDB) or the Lhasa Carcinogenicity Database (LCDB). However, many studies are not robust and cannot be solely relied upon for calculating AI limits. When the mutagenic potential of an NDSRI is not adequately characterized, the FDA and applicants have used (quantitative) Structure-Activity Relationship (SAR) methods to identify robustly tested surrogates that are structurally and reactively like the NDSRI. This surrogate's test data are then used to generate an estimate for the data-poor compound, a process known as read-across analysis. The selection of appropriate reference compounds for a read-across analysis considers factors such as the degree of substitution, steric bulk, electronic influences, potential for metabolic activation, stability/reactivity of the resulting metabolites, and overall molecular weight.

The U.S. Food and Drug Administration (FDA) has provided recommended acceptable intake (AI) limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs). These limits are based on their predicted carcinogenic potency categorization. The FDA's website includes recommended AI limits for 251 NDSRIs ranging from 26.5 ng/day to 1,500 ng/day. These limits are established based on rigorous scientific assessments and are intended to protect public health.

* For products intended for marketing in the United States, the FDA recommends an AI limit of 26.5 ng/day for Category 1, even if a different limit is recommended in other regulatory regions.

** See the guidance for industry Control of Nitrosamine Impurities in Human Drugs (February 2021).

*** See the International Council for Harmonisation guidance for industry M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (July 2023) (ICH M7(R2)). The threshold of toxicological concern (TTC) of 1.5 micrograms/day (1500 ng/day) as explained in ICH M7(R2), represents an AI for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effect.

Sources of nitrosamine impurities in drug products other than API contamination

Table 4: Sources of nitrosamine impurities in drug products other than API contamination (Cioc et al., 2023):

S. No.	Source of nitrosamine impurities	Remark
1	Raw materials	Nitrate and nitrite are the common sources of excipients that cause to generation of nitrosamine impurities.
2	Equipment's	Carryover from one product to another product improper cleaning or cleaning methods not in place. Hence this will lead to generating the nitrosamine impurities.
3	Utilities	Water is also the major source of nitrosamine impurities like water used for cleaning purposes and water used during drug product manufacturing.
4	Packing materials	Packing materials are also a major source of nitrosamine impurities source.
5	Degradation	Degradation formed due to incompatibilities of the API with the raw materials.

Prevention of nitrosamine contamination:

Nitrosating agents reacting with secondary, quaternary, or tertiary ammonium salt are used in different manufacturing processes of drug substances. Hence avoiding this reagent can prevent nitrosamine impurity formation.

Some Nitrosamine impurities are purged with solvent. The purged recovered solvent is reused and will be changed to increase the nitrosamine impurity. Hence they avoid the recovered solvent use in the manufacturing process. Also, the recovered catalysts may contaminate the nitrosamine impurities.

Contaminated raw materials, reagents and intermediates used in the drug manufacturing process are also major sources of nitrosamine impurities.

Equipment used for manufacturing of drug substance are cross-contaminated with the other product by improper cleaning of the equipment. Hence proper cleaning of the equipment should be in place.

Risk evaluation by considering the different stages like synthetic pathway, starting materials, intermediate materials, raw materials (Different solvents, catalysts etc.) and finished manufacturing process of the product (Law materials, packing materials etc.) will help to identify the source of the nitrosamine impurities in the API and drug product.

Regulatory Requirements and Recommendations

Nitrosamine impurities in pharmaceutical products have emerged as a significant apprehension due to their potential carcinogenicity. Regulatory bodies such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) have issued guidelines to control the presence of nitrosamine impurities34. The EMA has provided guidance to marketing authorization holders on how to avoid the presence of nitrosamine impurities[3].

Similarly, the FDA has recommended steps for manufacturers to detect and prevent unacceptable levels of nitrosamine impurities in pharmaceutical products.

In addition to these regulatory guidelines, scientific literature provides valuable insights into the risk factors and mitigation strategies for nitrosamine formation in pharmaceutical dosage forms. A comprehensive understanding of these factors is crucial for the development of effective control strategies.

Furthermore, the United States Pharmacopeia (USP) has developed documentary standards and a comprehensive portfolio of USP Reference Standards for nitrosamine impurities[5]. These standards provide a benchmark for impurity analysis and contribute to the overall quality assurance of pharmaceutical products. A detailed exploration of regulatory landscapes, including requirements and recommendations from global authorities such as EMA, FDA, and USP, sets the stage for effective risk management. The article emphasizes the need for proactive measures in compliance with evolving regulatory standards.

Case Study: Recalled Angiotensin II Receptor Blockers (ARBs)

The recall of Angiotensin II receptor blockers (ARBs) due to nitrosamine contamination serves as a compelling case study. This incident underscores the critical importance of stringent quality control in pharmaceutical manufacturing. ARBs, widely prescribed for conditions such as hypertension and heart failure, were found to be contaminated with nitrosamines, a class of probable human carcinogens. The discovery led to a global recall of affected ARB products, causing significant disruption in healthcare systems and raising concerns among millions of patients worldwide. This case not only highlights the potential public health risks associated with pharmaceutical contaminants but also underscores the need for robust detection methods and regulatory oversight to prevent such incidents. The lessons learned from the ARB recall are instrumental in guiding future efforts to ensure drug safety and protect public health.

Table 5: List of formulations recalled from the USFDA market due to the risk of nitrosamine impurities: USFDA recalled medicines(Buschmann, Handler, Holzgrabe, & Analysis, 2023)

S. No.	Brand-Names	Product-Description	Recall-Reason-Description	Company-Name
1	CHANTIX	Varenicline tablets	N-nitroso-varenicline above acceptable daily intake level	Pfizer
2	Lupin	SYMJEPI (epinephrine) Injection 0.15 mg (0.15 mg/0.3 mL) and 0.3 mg (0.3 mg/0.3 mL) Pre-Filled Single-Dose Syringes	API batches above the specification limit for the impurity, N-nitroso irbesartan	Lupin Pharmaceuticals, Inc.
3	Adamis Pharmaceuticals Corporation	Metformin Hydrochloride Extended-Release Tablets	Potential clogging of the needle prevents the dispensing of epinephrine	Adamis Pharmaceuticals Corporation

4	Viona	Senna Syrup 5mL	N-Nitrosodimethylamine (NDMA) Impurity	Viona Pharma5ceuticals, Inc.
5	Macleods	All Day Hand Sanitizer	Presence of NMBA impurity	Macleods Pharmaceutical Limited
6	Lupin	Hand Sanitizer	N-Nitrosodimethylamine (NDMA) Impurity	Lupin Pharmaceuticals, Inc.
7	Viona Pharmaceuticals Inc.	R.E.C.K. (Ropivacaine, Epinephrine, Clonidine, Ketorolac) 50 ml in Sodium Chloride-60 ml BD syringe	Contains Nitrosodimethylamine (NDMA) impurities	Viona Pharmaceuticals Inc.
8	Gemini Laboratories	Ranitidine Liquid Unit Dose Cups	NDMA (Nitrosodimethylamine) impurity	Amneal Pharmaceuticals, LLC
9	Lupin	Metformin HCl Extended-Release Tablets, USP 750 mg	Detection of N-Nitrosodimethylamine (NDMA)	Lupin Pharmaceuticals, Inc
10	AHP	Thyroid Tablets	NDMA (Nitrosodimethylamine) impurity	American Health Packaging
11	PrecisionDose	Metformin Hydrochloride for Extended-Release Oral Suspension	The potential presence of N-Nitrosodimethylamine (NDMA) above levels established by the FDA	Precision Dose Inc.
12	Nostrum Laboratories	CORE essential nutrients and Immune Boost Sublingual Vitamin D3	NDMA exceeds the acceptable daily intake limit	Nostrum Laboratories
13	Nostrum Laboratories, Inc.	Sodium bicarbonate injection USP	Due to levels of nitrosamine impurities above the ADI limit of 96 ng/day	Nostrum Laboratories, Inc.
14	Riomet ER	Puriton Eye Relief Drops, 0.5 oz. (15ml) bottle	Due to the Detection of N-Nitrosodimethylamine (NDMA) Impurity	Sun Pharmaceutical Industries
15	Nostrum Laboratories	Drospirenone and Ethinyl Estradiol Tablets, USP, 3 mg/ 0.02mg	NDMA exceeds the acceptable daily intake limit	Nostrum Laboratories
16	Mylan	Homeopathic Medicines	NDMA (Nitrosodimethylamine) impurity	Mylan N.V.

17	Sandoz Inc.	Nitroglycerin Lingual Spray	Due to an Elevated Amount of Unexpected Impurity, N-Nitrosodimethylamine (NDMA)	Sandoz Inc.
18	Northwind	Dietary Supplements with undeclared Diclofenac	NDMA (Nitrosodimethylamine) impurity	Denton Pharma, Inc. dba Northwind Pharmaceuticals

Conclusion

It is concluded the case of nitrosamine contamination in angiotensin II receptor blockers undergoes and has a very crucial importance of continuous alert mode and stringent stick to regulatory quality in the pharmaceuticals industry. The incident serves as a stark reminder of the potential public health risks related to pharmaceutical impurities. It highlights the need the robust detection methods, stringent quality control measures, and proactive regulatory oversight to prevent such occurrences. The recall of ARBs due to nitrosamine contamination has not only disrupted healthcare systems worldwide but also raised significant concerns among patients. However, it has also provided valuable lessons that are instrumental in guiding future efforts to ensure drug safety and protect public health. As we move forward, these lessons must inform our strategies and actions in the pursuit of pharmaceutical safety and efficacy. The significance of this case study extends beyond ARBs, reminding us of the broader implications for the pharmaceutical industry and the necessity for ongoing vigilance in our collective commitment to patient safety.

Conflict of Interest

The authors declare no conflict of interest.

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Abbreviations

DNA: Deoxyribonucleic Acid, APIs: Active Pharmaceutical Ingredients, ARBs: Angiotensin II Receptor Blockers, EMA: European Medicines Agency, FDA: Food and Drug Administration
USP: United States Pharmacopeia, IARC: International Agency for Research on Cancer, NDMA: N-Nitrosodimethylamine, NDEA: N-Nitrosodiethylamine, GSRS: Global Substance Registration System, TTC: Threshold of Toxicological Concern, LCDB: Lhasa Carcinogenicity Database, AI: Acceptable Intake, NMBA: N-Nitrosomethylaminobutyric Acid, NEIPA: N-Nitrosoethylisopropylamine
NDIPA: N-Nitrosodiisopropylamine, NDPA: N-Nitrosodipropylamine, NDBA: N-Nitrosodibutylamine, NPIP: N-nitrosopiperidine, LOQ: limit of Quantification, LOD: Limit of Detection, MDD: Maximum Daily Dose, ESI HRMS: Electrospray Ionization High-Resolution Mass Spectrometry, GC: Gas Chromatography, MS: Mass Spectrometry, TFDA: Taiwan Food and Drug Administration, HSA: Health Science Authority, APCI: Atmospheric Pressure Chemical Ionization, DP: Drug Products, DS: Drug Substance, NDRSIs: N-Nitroso Dimethylamine-Related Substances

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