Nitrosamine Impurities In Pharmaceuticals: A Comprehensive Review

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ABSTRACT
When several nitrosamine impurities are found and their cumulative amount surpasses 26.5 ng/day (which is the maximum daily dose (MDD) allowable consumption for the nitrosamine), the FDA asks the producers to get in touch with them so they may conduct an assessment. When evaluating the risk of human cancer, controlling potentially mutagenic contaminants in pharmaceutical products and crucial interest in the mutagenic and carcinogenic potential of nitrosamine impurities has grown since they were recently found in several medicinal products that are commercialized. A "Cohort of Concern" for which the chemical class is deemed to belong indicates that some common control procedures, including using the threshold of toxicological concern (TTC), cannot be used.
These contaminants were in the pharmaceutical products throughout the production process via raw materials, catalysts, and solvents. By altering the production process or taking safety measures while producing drug products, nitrosamine impurities can be prevented.
To identify and characterize these contaminants validated analytical methods are applied. Mass spectrometry, liquid chromatography, and gas chromatography are the analytical techniques. These impurities originated from the first time a nitrosating agent was used in secondary, tertiary, and ammonium salt. These techniques aid in maintaining a low concentration of nitrosamine contaminants in a drug or drug product intended for use in human medicine.

Keywords FDA, TTC, nitrosating, mass spectrometry, liquid chromatography, gas chromatography

INTRODUCTION

Nitrites and other nitrogen-containing compounds react with secondary carbamates of amides, amines, and urea derivatives to generate nitrosamine.
The oxidation state of nitrogen is +3. A drug may include nitrosamine for several reasons. The production and packaging of pharmaceuticals is the source of nitrosamine.
The identification of nitrosamine, a likely human carcinogen, in medications like metformin, ranitidine, nizatidine, and angiotensin II receptor antagonists (ARBs) has amply illustrated the necessity of creating a risk assessment plan for any pharmaceutical product that may contain nitrosamines and the degree of risk that comes with their presence.
The FDA and other foreign regulatory bodies conducted a thorough investigation of these contaminants in the impacted active ingredients and pharmaceutical products when nitrosamines were found in them.

1. Assessing the risks associated with both marketed or authorized goods and those for which applications are still pending.
2. Take the necessary actions to lessen or eliminate the nitrosamines found in active components and pharmaceutical goods.

Nitrosamine may be present in other active ingredients and pharmaceutical products due to the use of processes and materials that may be sensitive to nitrosamine. Even though nitrosamine has only been detected in certain pharmaceutical products many of these products have been recalled due to unacceptable levels of these nitrosamines.
We have general methods for detecting nitrosamine impurities: GC-MS, LC-MS/MS[1]
Nevertheless, although the FDA has been looking into nitrosamine contaminants in pharmaceutical products since June 2018, it has only recently started to concentrate on NDSRIs, a class of nitrosamine that is structurally related to an active ingredient in pharmaceutical products.
In November 20221, the FDA released an update on potential corrective measures and tactics to lower the risk of drug-related nitrosamine contamination in pharmaceutical products, making it the first agency to report on the NDSRI industry presence.
NDSRIs are produced, synthesized, and stored as pharmaceuticals. These compounds are not the same as low molecular weight nitrosamine pollutants listed in the FDA's nitrosamine guidance.
Taking into account the possibility of forming the NDSRI on a parts per million bases.
Nitrite impurity contents (found in water and frequently used excipients, for example). There are known medications that carry the risk of nitrosamine formation at this time[2]

Because some of the chemicals in this structural class have a high potential for cancer, N-nitrosamines are included in the cohort of concerns (COC). N-nitrosamines are one of several structural classes identified by the ICH M7 guideline, which offers a framework for security assessment and control of mutagenic impurities in pharmaceutical products. This is probably going to be the acceptable intake limit (AI) for non-COC carcinogens, which is significantly lower than the exposure limits or threshold toxicological concerns (TTC). Not every N-nitrosamines, though is a potent carcinogen. For instance, when data on the carcinogenicity of N-nitrosamines was gathered for 228 low molecular weight derivatives, it was discovered that 18% of N-nitrosamines were not carcinogenic to animals. In addition, for the carcinogenic N-nitrosamine, the logarithmic TD_{50} values are approximately four orders of magnitude higher and overlap the carcinogens with non-nitrosamine content, such as those that are not included in Category COC[3]

The FDA has looked into claims that certain pharmaceutical products contain nitrosamine contaminants. Several drugs had unacceptable amounts of nitrosamines in 2018, including metformin, ranitidine, nizatidine, and ARBs.

The FDA learned in September 2019 that certain well-known heartburn medications (nizatidine better known as Axid and ranitidine commonly known as Zantac) contained excessive amounts of NDMA.

The FDA discovered in December 2019 that certain metformin-containing diabetes drugs also contained NDMA from other nations. With this knowledge, the FDA ordered metformin samples to check for the presence of NDMA. The agency detected in certain samples in February 2020, but the concentration was not found to be higher than the allowable absorption limit. An additional FDA investigation conducted in May 2020 discovered that certain lots of extended-release metformin had levels of NDMA above the suggested acceptable intake limit[4]

On May 4, 2023, the FDA recommended manufacturers the same engineering-identified processes in their first note to the industry about the presence of NDSRI. Current FDA nitrosamine identification guidelines for NDSRI presence[2]

PHOTOCHEMISTRY

Photolysis: Since the 1930s, it has been established that NDMA and other N-nitrosamines are exposed to UV radiation which fragments their N-N compound. NDMA excitation causes photolytic cleavage of an excited single state via its $\pi \rightarrow \pi^*$ (S0 →S2) or $n \rightarrow \pi^*$ (S0 →S1) transition. The nitrogen-produced nitric oxide (NO) and dimethylamine residue can then recombine to form the original state[5]

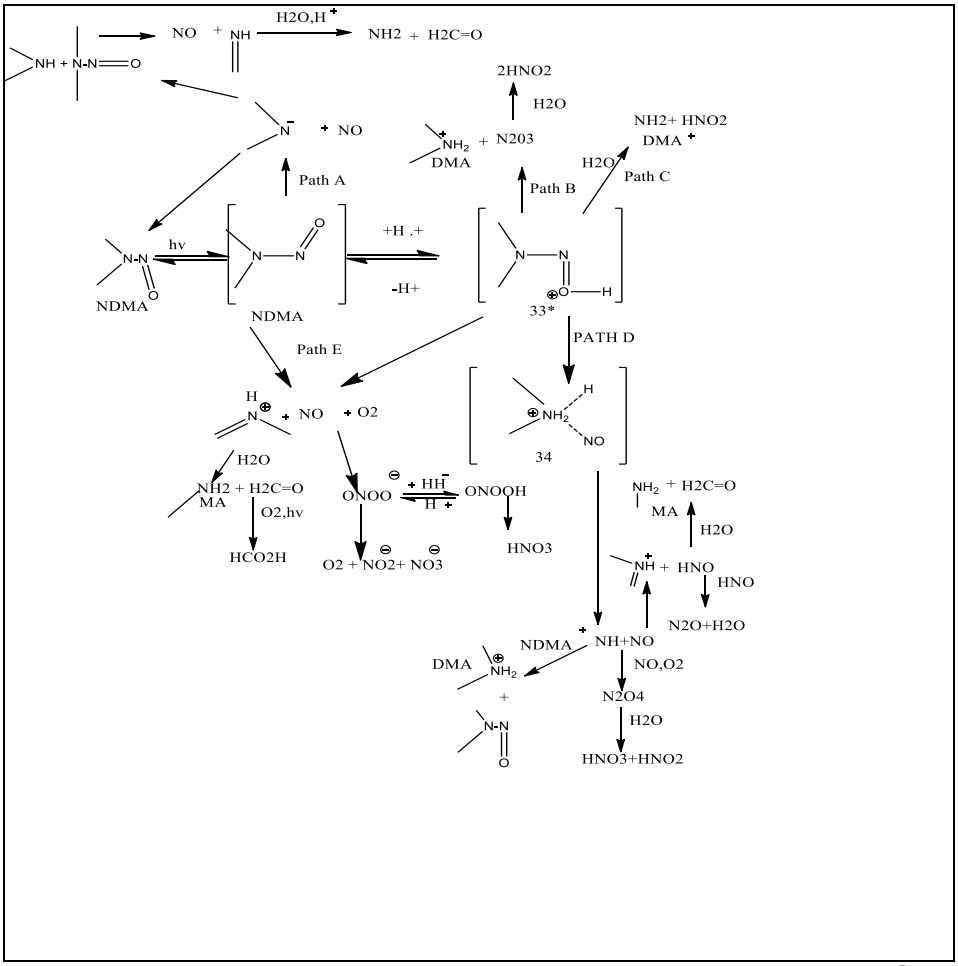
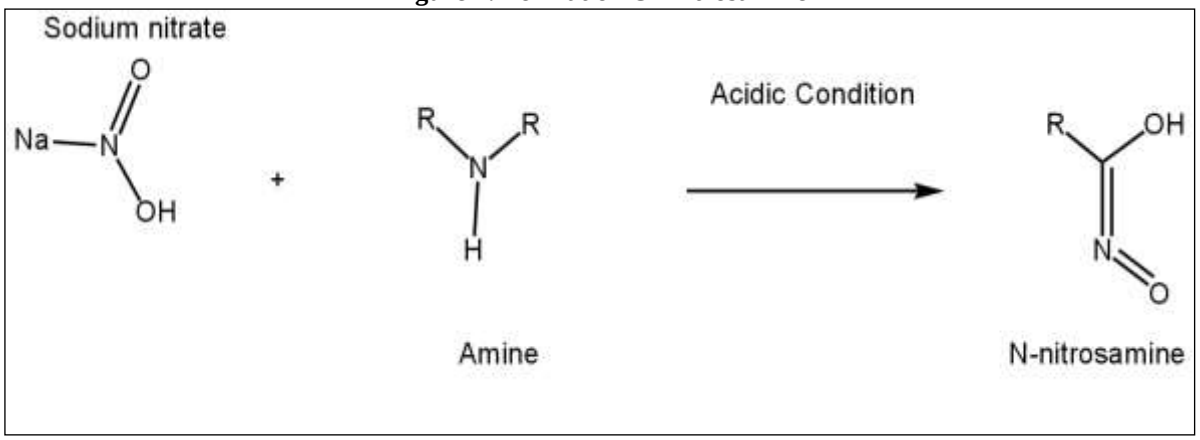


Figure 1: Mechanism of N-nitrosamine photolysis in solution using NDMA[5]

FORMATION OF NITROSAMINE

Figure 2: Formation Of Nitrosamine



OLIGONUCLEOTIDE DERIVED NITROSAMINE

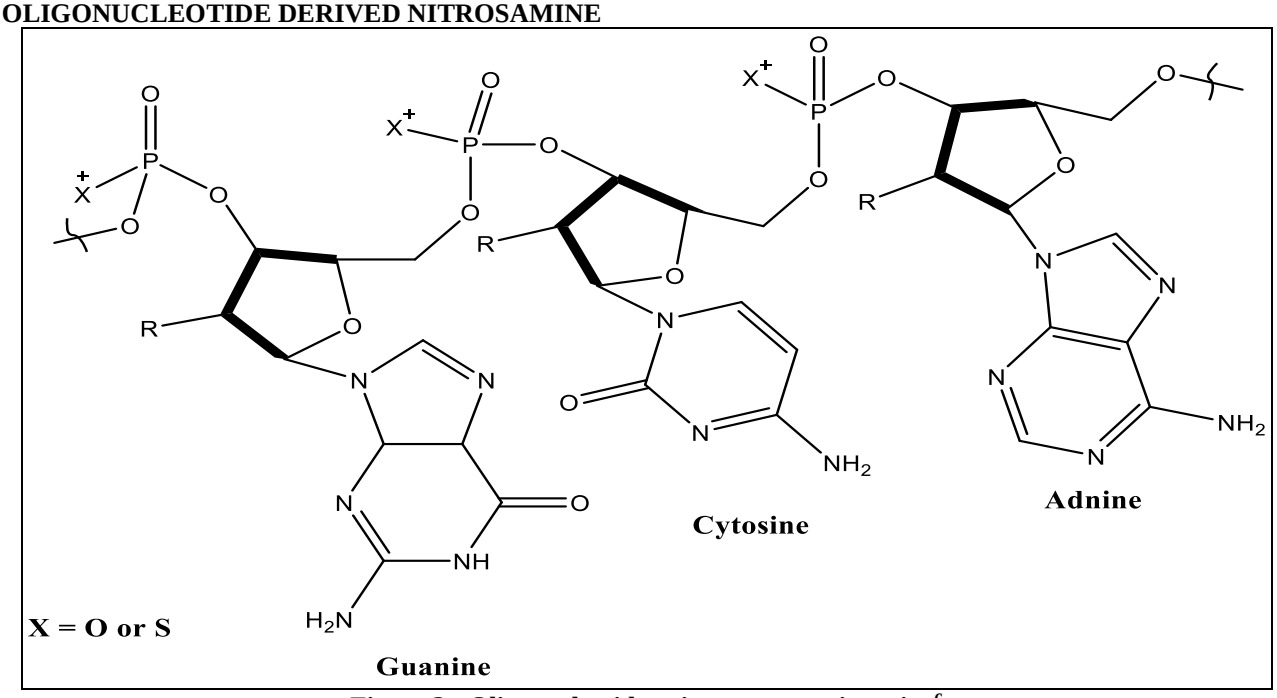


Figure 3: Oligonucleotide primary aromatic amine[6]

SOURCES OF NITROSAMINE

Secondary or tertiary amines react with nitrites in an acidic environment to produce nitrosamines. Nitrosamines can be added to medications or made as additives in a variety of ways.

The API itself has the potential to degrade and release nitrosamines (like ranitidine) in specific situations. Dialkyl amines are generated when solvents like dimethylformamide (DMF) break down. Impurities in catalysts, raw materials, reagents, solvents (including recycled solvents), or other substances.

Impurities in the solvents, reagents, intermediates, and starting materials used in their preparation. Impurities present in the water, excipients, or processing aids used to make the pharmaceutical product in its final form. When the medication is made in the presence of the precursors required for the synthesis of nitrosamines and under specific reaction conditions. Impurities may form nitrosamines in the packaging closure system of the completed drug product, especially if they are linked to materials that contain amines and possible sources of nitrosating agents (nitrocellulose, nitrite, etc)[7]

ROOT CAUSES FOR NITROSAMINES IN APIs

1. General circumstances that cause the formation of nitrosamines.
2. Secondary, tertiary, and quaternary amine sources that can combine to produce nitrosamines.
3. Pollution in raw materials obtained from vendors.
4. Solvents, catalysts, and reagents were identified as potential sources of nitrosamines.
5. Nitrosamine can be obtained from the quenching process.
6. Inadequate control and optimization of the process.[8]

ROOT CAUSES FOR NITROSAMINES IN MEDICINAL PRODUCTS

1. Environment

N-nitrosamines are produced and found in the environment. They are mostly created in trace amounts in water during biological processes and airborne combustion processes. As low as reasonably practicable (ALARP) or as low as reasonably achievable (ALARA) are the guiding concepts of control strategies. Concentrations are thought to be higher in places with less regulation as well as high pollution levels in air and water.[8]

2. Food

In foods, the reaction of nitrites and amines, which can produce nitrosamines at higher temperatures in meat, fish, and other products, is the main cause of N-nitrosamine formation. Restricting the use of nitrates and nitrites in food production is one way to minimize formation.[8]

The European Food Safety Authority (EFSA) confirmed this by citing several studies that demonstrated that the primary cause of the total external exposure ranged from 0.2 ng/kg/day for newborns to 3.5 ng/kg/day for small children and was caused by exposure to volatile N-nitrosamines (NDMA plus NDEA) from processed meat. N-nitroso dimethylamine (NDMA), N-nitroso diethylamine (NDEA), NDIPA, and NDPA were all analyzed in unpublished data on the actual nitrosamine content of cooked and processed foods, however, only NDMA (and NPIP) at 0.7 to 0.9 ppb or 14 to 17 ng/day consumption levels were found. Based on the ALARA principles, regulations to reduce the formation of N-nitrosamines in food, beverages, and beer are designed to limit exposure (EFSA 2017).[8]

3. Drinking Water

The German Umweltbundesamt (UBA) has set a limit value of 10 ng/l for NDMA in drinking water. This level is thought to pose a health risk for both short-term and long-term exposure. New Jersey has set a public health goal of 0.7 ng/l for NDMA and 5 ng/l for NDPA in groundwater, while California has set a goal of 3 ng/l for drinking water. Health reference levels for NMBA (30 ng/l), NDEA (0.6 ng/l), NDPA (7 ng/l), NMEA (3 ng/l), and NPYR (2 ng/l) have been set by Environmental Protection Agency (EPA).[8]

IMPACT ON EXCIPIENT TYPE AND SUPPLIER ON NITROSAMINE FORMATION

Based on known values, a set of case studies was created to determine the theoretical amount of nitrosamine that would form in each tablet:

- The amount of nitrite in every excipient.
- The molecular mass of the nitrosamine that is going to form.
- Weight of tablet dosage.
- Anticipated percentage of nitrite to nitrosamine conversion.
- How the dosage form is made.

Based on nitrite levels and conversion rates, the potential nitrosamine formation (ppm) as per equation 1 for each excipient in each case study was determined. Next, the final nitrosamine content and tablet composition were used to calculate the excipient's deployment. In terms of total nitrosamine formation, the effects of average, maximum, and minimum nitrite values on particular excipients were compared.[9]

$$\text{Per excipient, nitrosamine (ppm)} = \text{nitrite level in excipient(ppm)} \times \text{MW of nitrosamine(g/mol)} \times \% \text{ of conversion/MW of nitrite}$$

TYPES OF DRUG AFFECT

As of the most recent update, an evaluation is being carried out by the Committee of Human Medicines (CHMP) of the European Medicine Agency (EMA) to determine whether certain batches of ranitidine contain the nitrosamine known as NDMA. Gather information and learn how NDMA was found in these batches of ranitidine. Additionally, the EMA is evaluating the fallout from recent tests that found NDMA in some batches of metformin, a medication used to treat diabetes, in coordination with national authorities. The evaluation's goal is to determine the degree and possible hazards connected to NDMA's presence in these metformin batches. These continuous evaluations and assessments aim to gather all pertinent data, look into the origin of NDMA contamination with metformin and ranitidine, and assess the possible influence on patient security. The European Medicine Agency (EMA) and national authorities are dedicated to implementing the necessary steps to mitigate all hazards identified and safeguard patient's health and welfare.[10]

METABOLIC ACTIVATION OF NITROSAMINES

N-nitrosamines are typically regarded as indirect mutagens that necessitate hydroxylation of the α-carbon atom on the nitroso residue to activate cytochrome P-450. Certain reports indicate that the initial α-hydroxylation reaction involves free radical mechanisms. The next mutagen is thought to be alkyl diazonium ion, which is produced by other reactions.[11] After N-nitrosamines were incubated with a reconstituted monooxygenase system made up of cytochrome P-450 and NADPH P-450 reductase, pig liver was used to produce nitrite. When nitrosamines were incubated with NADPH P-450 reductase on its own or without the presence of molecular oxygen or NADPH, no nitrite was produced. When nitrosamines interacted with hemoglobin under reducing conditions or the reconstituted P-450 system, the resulting optical spectra were the same as those for nitrite. It is thought that electrons denitrosate N-nitrosamines.[12]

INVESTIGATION AND SYNTHESIS OF NDSRIS

From the initial focus on the potential presence of known small molecules of N-nitrosamine, such as N-nitroso diethylamine (NDEA), in active substances, NDSRIs in medicines, have been the shift in the risk assessment of N-nitrosamine in medicinal products. Simple secondary amines can be easily N-nitrosed, but more complex amines can follow different reaction pathways that are harder to predict. It is known that a large number of these complex amines do not undergo N-nitrosation; instead, they are either unreactive or react through different pathways. C-nitrosation or nitration, to yield products other than N-nitrosamine. To investigate the possibility of creating new N-nitrosamines through the nitrosation of complex amines, a standard set of three reaction conditions for forced degradation via orthogonal nitrosation is available. In light of risk factors in manufacturing, it is thought that these complementary reaction conditions allow an accurate assessment of the potential N-nitrosamine formation from complex amines. If after researching nitrosation under the suggested circumstances, it is not possible to form and isolate N-nitrosamine, the knowledge gained about chemical stability and reactivity can be used to support the idea that a specific N-nitrosamine should not be a drug substance or product. The information gathered can be utilized as part of the risk assessment if N-nitrosamine is produced under these reaction circumstances and serve as a springboard for creating a procedure to assemble an alternative sample for further analysis. Additionally, when creating novel N-nitrosamine for use in toxicological or analytical research, synthetic and analytical factors need to be taken into account.[13]

CANCER RISK ESTIMATE

By using the US FDA's reported nitrosamine levels, we were able to calculate cancer risk and estimate the possible consequences of taking drugs tainted with nitrosamine. While many drugs have been found to contain NDMA and NDEA, only a small number of drugs have reported contaminant levels, and even then the levels varied amongst the drugs. In regards to NMBA, it has been found in products that contain valsartan and losartan, however, no amounts of NMBA have been documented in these products. The valsartan product had the highest NDEA value of 1.31 µg per tablet and the highest NDMA value of 20.19 µg per tablet, as reported by the US FDA. The US FDA's interim acceptable intake limit of 0.096 µg/day for NDMA and 0.0265 µg/day for NDEA.[14]

N-NITROSAMINES IMPACTING DRUG INTERACTION STUDIES

In a clinical setting, patients who take two or more medications concurrently may experience drug-drug interactions (DDIs), which could compromise patient safety or reduce the effectiveness of medications. For instance, CYP liver enzymes, drug transporter inhibitory molecules, or inductive drugs may change absorption, and the elimination of additional medications may cause the victim's drug levels to lie above or below therapeutic ranges. The ICH has released guidelines requiring the clinical significance of these risks to prevent unanticipated safety risks. During the development of these new medications, DDIs will be compared between study medications and other medications. Studies aiming at evaluating the potential pharmacokinetic sensitivity of new drugs, especially small molecules under development, are usually focused on the experimental drugs' metabolic pathway or possible impact on a vital enzyme and carrier.

The EMA and FDA published guidelines in 2020 and 2021, respectively, directing the industry to check human drugs for N-nitrosamine impurities. These guidelines are responsible for the increased monitoring of medications that have led to the current findings on N-nitrosamine contamination.

Because nitrosamine control in pharmaceutical products is so important, recent in silico analysis has shown that several DDI inhibitors and substrates are possible precursors of N-nitrosamines and are therefore likely candidates for the synthesis of NDSRI.[15]

ACCEPTABLE INTAKE LIMIT OF NITROSAMINE IN DRUG PRODUCTS

Table 1 Acceptable Intake Limit[1]

Nitrosamine	Limit (ng/day)
NDMA	96
NDEA	26.5
NDIPA	26.5
NIPEA	26.5
NDBA	26.5

DRUG PRODUCT RECALL

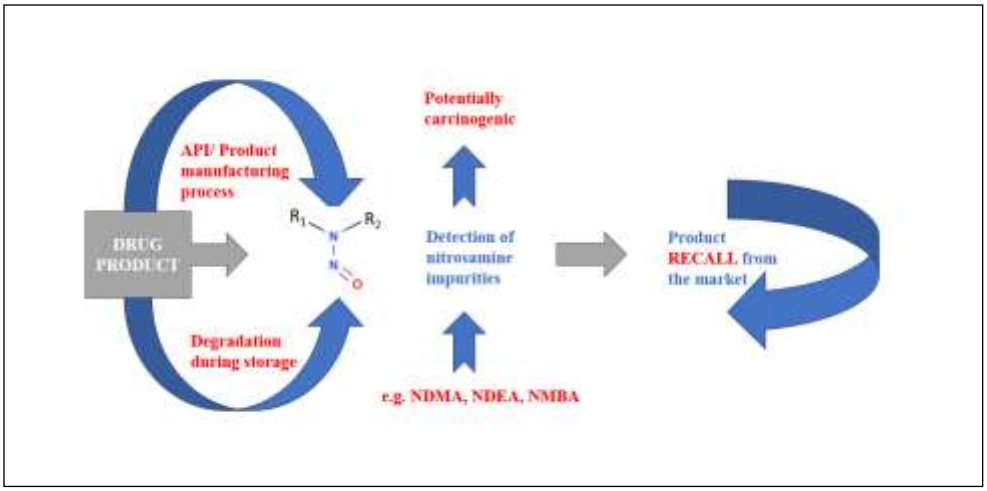


Figure 4 Drug Product Recall[16]

INHIBITORS INVESTIGATED

1. Ascorbic Acid

Like all nitrosating agents, ascorbic acid has a wide range of applications and is non-toxic when used in dosages that are effective for the drug. It is especially well suited for aqueous and mildly acidic environments, where it can react with N_2O_3, $H_2NO_2^+$, and NOX to produce NO. but in an aerobic environment, NO can be transformed back into nitrosating agents (like N_2O_3 or N_2O_4) by oxidizing to NO_2. As a result, even though redox oxides like ascorbic acid have a very high removal efficiency is anticipated in aerobic conditions. Pharmaceutical products that contain ascorbic acid in the proper concentration effectively prevent the formation of N-nitrosamine from the amount of nitrosating agent expected under storage conditions that lead to the formation of N-nitrosamine in aqueous solution. When it comes to solid dosage forms made by direct compression, this method should be used carefully though, as the effects of particle size and local distribution on capture kinetics can affect how effective the inhibition is. The scavenger is guaranteed to be in close proximity to the nitrosating agent throughout the drug product matrix due to the smaller particle size and uniform distribution issues with particle size and uniformity can be lessened with wet granulation.[17,18]

2. α- Tocopherol

N-nitrosamine formation is inhibited by the antioxidant activity of α-tocopherol. Similar to ascorbic acid, α-tocopherol can convert the nitrosating agent into a non-nitrosating compound, thereby posing a competition for the nitrosating agent with sensitive amines. Since the aromatic ring of α-tocopherol is fully substituted. C-nitroso is insignificant even though all tocopherols have a phenol ring that can reduce the nitrosating agent. The formation of N-nitrosamine can only be inhibited by the non-esterified form of α-tocopherol, as oxidation produces radicals that form the hydroxyl group. When α-tocopherol reacts with a nitrosating agent, αtocopherol quinone is created. While α-tocopherol has been shown to work well in aqueous media, it is more effective in lipophilic media due to its lipid phase solubility and low water solubility. On the other hand, the nitrosating substances nitric anhydride (N_2O_3) and dinitrogen tetroxide (N_2O_4) are lipophilic, making it crucial to consider both the active ingredient's lipophilicity and the presence of the nitrosating substances.[17,18]

3. Amino Acids

Primary amines and thiol groups found in amino acids make them nitrite scavengers. The fundamental mechanism involves the diazotization reaction with a nitrosating agent to form diazo intermediates. These intermediates are unstable and quickly transform into nitrogen and deamination products (like alcohols) after the Van Slyke reaction, which can fragment all amino acids except proline.[17,18]

4. Other Compounds

Additional substances have the potential to be used as adjuvants for the attenuation of N-nitrosamine. Ascorbic acid and α-tocopherol have been studied more than these, but they frequently have an antioxidant effect. Examples of these include maltol and propyl gallate, resveratrol, butylated hydroxyanisole (BHA) butylated hydroxytoluene (BHT), para-aminobenzoic acid (PABA), and caffeic and ferulic acid. When given as a solid dosage form, the model amine 4-phenyl piperidine hydrochloride is dissolved at a concentration of 0.1% w/w ferulic acid and caffeic acid both inhibit the formation of N-nitrosamine by 60%, while caffeic acid completely prevents it.[17,18]

MUTAGENICITY AND CARCINOGENICITY DATA

Vitic is a structured, searchable database that is available for purchase and contains toxicological data that is primarily obtained from public sources (Lhasa Limited, 2020). Several literature searches were carried out using PubMed (the U.S. National Library of Medicine (NLM)) to provide a list of publications containing nitrosamine test data to increase the coverage of nitrosamines in Vitic. With a 35% increase in Ames test data, the number of nitrosamines in the database increased by 24%.[19]

DETECTING NITROSAMINE IN PHARMACEUTICAL PRODUCTS

Many laboratories are looking for instruments and methods that allow accurate identification and quantification of nitrosamines because of growing concerns and regulatory requirements. Liquid Chromatography (LC) MS and gas chromatography-mass spectrometry (GC-MS) are currently recognized techniques for testing small molecule drugs. These techniques allow for the detection, identification, and quantification of compounds; however, the equipment needed to meet the necessary detection limits is frequently costly, and interfering compounds can result in false positive results. The pharmaceutical manufacturing process poses significant challenges for the easy integration of analytical techniques due to its highly regulated nature.

Using a Thermal Energy Analyzer (TEA) detection system for highly specific chemiluminescence detection is an alternative detection method that is becoming more and more popular. It is simple to connect to the GC and with the appropriate hardware interface to the LC. Since its development in the late 1960s, TEA has been the industry standard for nitrosamine analysis in many other industries due to its ability to quickly identify and analyze N-nitrosamine compounds. The matrix effect is less of an issue with TEA's selectivity for Nitroso-compounds than it is with MS detection.[20]

ANALYTICAL CHALLENGES

Based on the maximum daily API dose and the tolerable NDSRI dose, analytical methods must have a limit of quantification (LOQ) of 10% of the authorized level to prove the absence of NDSRI. NDSRI quantification is thought to be routinely achievable at concentrations of up to 50-100 ppb. Low allowable intake levels combined with acceptable, even moderate, daily doses of API. However, this may result in LOQ requirements that exceed the go-beyond feasibility technology for NDSRIs for which the CPCA requires control in categories 1 through 3. When an NDSRI and an API are co-eluted, the analytical difficulty may rise because of the strong physicochemical similarity or the absence of reliable reference standards.

More sensitive results can be obtained when these are present and co-elution with the API does not present any issues. As an illustration, it has been stated that NDMA can be measured down to 1 ppb; however, this is an anomaly and cannot be routinely replicated in a laboratory for quality control purposes.[21]

MOLECULAR EPIDEMIOLOGY OF NITROSAMINES

In vitro and in vivo testing are frequently used to assess a chemical's carcinogenicity. Animal studies in particular can yield dose-response data that help forecast human reactions. It is crucial to stress, though that epidemiological data are regarded by regulatory bodies as the most solid proof of carcinogenicity in humans.

Molecular epidemiology, which integrates analytical epidemiology and molecular toxicology, has greatly advanced cancer research in academic and community contexts.

The fundamentals of molecular epidemiology involve tracking the exposure of humans to carcinogens, examining pre-clinical reactions in multistage chemical carcinogenesis, and measuring biomarkers in human tissues and body fluids, such as carcinogenic DNA adducts, metabolites, and other genotoxic alterations.[22]

HOW TO AVOID NITROSAMINE FORMATION

Nitrosamine levels in medications can vary depending on several factors. Anywhere in the process chain, from the synthesis of APIs to the production of pharmaceuticals and the storage of the final product, nitrosamines can form. Depending on the circumstances surrounding their creation, they may take one or more steps to form. It appears that the reduction happens in a significant amount of amines and/or nitrosation by the law of mass action. If it has an impact on the API, a reduction of the sensitive amine is undoubtedly not a feasible reduction of substances that are sensitive or nitrosate. By altering the denatured supplier material, the amine can be obtained. The contaminant (secondary amine or nitrosating agent) or the nitrosamine formed can be removed by extension or additional purification steps, or in the case of the nitrosamine, it decomposes due to its susceptibility to reducing, oxidizing, and electrophilic effects, nucleophiles, and radical chemistry. This is all under the control of the marketing authorization holder when it comes to API synthesis. However, because there aren't many options, switching suppliers might not be practical or significant. Changes to the API summary involve both approved business processes and ongoing development, making them intricate and time-consuming.

The amount of nitrosamine that forms in the formulation of pharmaceutical products can be decreased by raising the pH, adjusting the water content or particle size, or adding inhibitors. Once more, these steps can be taken as a fast fix for current business issues or problems with products that are far along in the development process. However, any modification made to the recipes should be thoroughly considered for any potential negative effects, or side effects from non-nitrosamines, such as stability, manufacturability, and physicochemical changes in properties. Process modification device settings or procedures that impact the impact of heat, such as if technically possible, the consideration of the humidity level.

Stricter storage guidelines and/or the use of primary packaging that provides protection can help minimize nitrosamine formation that may have occurred during the medication's storage. Longevity may be another good choice. Less developed nations may have limited or no access to cold storage and developing nations- particularly in private homes may have more expensive, more protective packaging. However, a shorter shelf life has no bearing on the patient's or the responsible party's convenience until it becomes unsustainable and adds to the burden on the populace. Supply continuity and environmental equilibrium.[23]

Precisely control and assess genotoxic contaminants in drug ingredients and drug product manufacturing processes, as well as formulation process development, validation, and degradation pathways. With the development of contemporary analytical techniques, such as risk assessments, critical process parameter (CPP) checks, and element identification, contamination can be systematically assessed and controlled using the principles of Quality by Design (QbD), Control Strategies, evaluation criteria, and variables to optimize.[24]

CONTROL STRATEGY

The applicant or holder of the marketing authorization must design the plan of action, considering both present and future steps to reduce the possibility of nitrosamine formation or contamination. These steps may include modifications to the manufacturing process, the use of particular test methods, and the creation of suitable test procedures, facilities, and equipment.

Conduct a risk assessment, taking into consideration the information that is currently available regarding the presence of N-nitrosamine in the medicinal product, to evaluate the production process (e.g. raw materials, starting materials, and synthetic route).

Perform a risk assessment for the completed dosage form, taking into account the sources of N-nitrosamines (excipients, primary packaging material, degradation of the API, etc).

MAH candidates are regularly checked by the appropriate authorities to make sure they are adhering to the aforementioned requirements.[25]

CONTROL OF NITROSAMINE

Step 1- Risk Assessment

Risk assessment is used to determine which active ingredients and products are most likely to cause N-nitrosamine contamination. It also reports the effects of biological and chemical drugs as of July 1, 2021, and March 31, 2021, respectively. Owners of marketing authorization should ideally move on to phase 2 of the final product verification test and submit a detailed response template. Marketing authorization holders are required to conduct a risk assessment of the product. If there is no obvious risk associated with the active ingredient. They should only be present in a single-stage result once the active ingredient and final product have been determined. Managers who have marketing authorization can email products to a group for notification.[1,26]

Step 2- Verification Test

If they have purchased nitrosamine for their product and satisfy at least one of the following requirements, authorized marketing authorities are required to use Step-2 nitrosamine received above the approved dose or the recently obtained nitrosamine response model:

a. Exceeds the reasonable acceptance threshold.
b. Outweights the cancer risk by a factor greater than 1:100,000.
c. Recently discovered nitrosamine is not covered by CHMP Article 5(3), regardless of the amount received.

In these situations, you also have to submit a Phase-2 response to nitrosamine detection with your submission.[1,26]

Step-3 Review the Marketing Authorization

By using standard control procedures to submit a marketing authorization change application, you can request any necessary modifications to the manufacturing process that arise from this evaluation. Holders of marketing authorizations are required to complete an additional application and pass a verification test by:

a) September 26, 2022, about pharmaceuticals.
b) July 1, 2023, regarding biologic medications.

Phase 2 of the measure, which attempts to accurately ensure the requirements for the authorized sale of sartan-based medications, which must be respected to avoid the presence of the nitrosamine impurities contained in their products are the same in all human medicines, must be carried out to respect these final days.[1,27,28]

RISK ASSESSMENT FOR NDSRIs

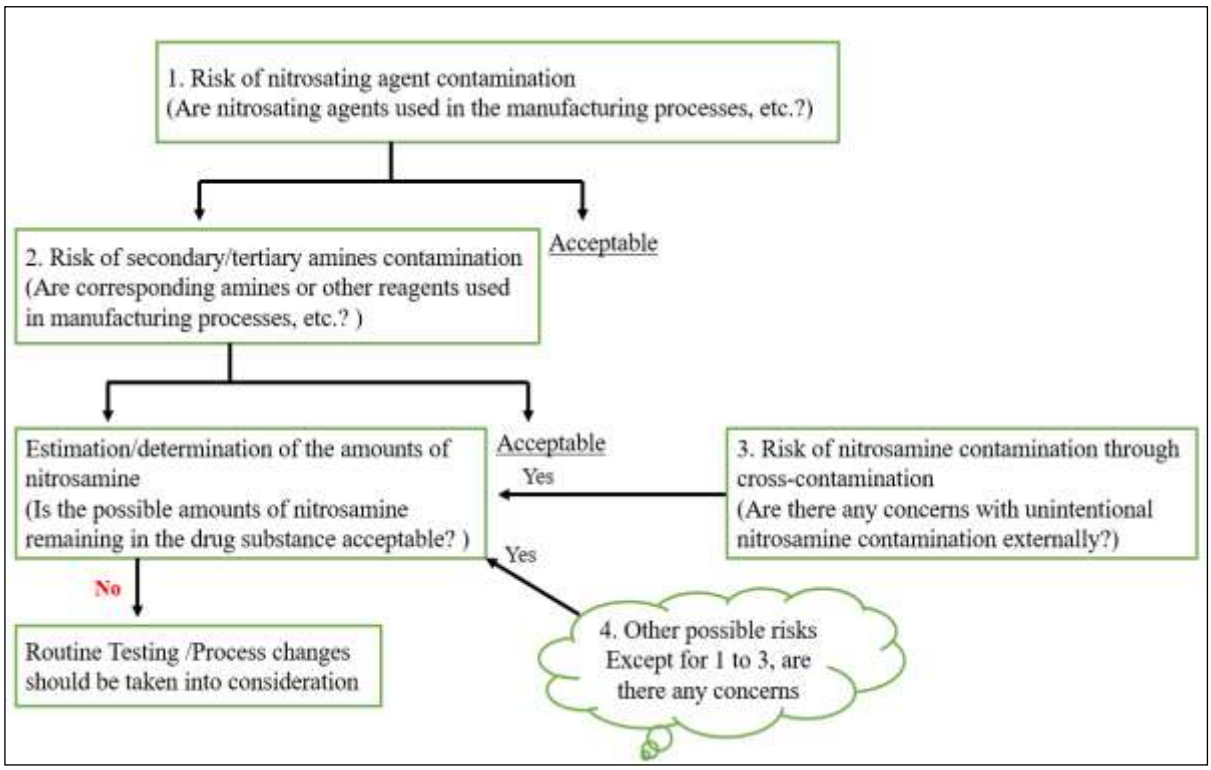


Figure 5: Risk Assessment of Nitrosamine Contamination in Drug Substances[29]

REVIEW OF MANUFACTURING PROCESS

Marketing authorization holders can use templates published by the European Medicine Agency (EMA) to submit tests for nitrosamine contamination of products. The following actions are requested by the manufacturer to regulate nitrosamine classification in human pharmaceuticals.[30]

Step 1- Risk Evaluation

Within six months, the manufacturer of the medicinal product and the holder of the marketing authorization must evaluate the risks associated with the use of nitrosamine in compliance with ICH Q9 and M7 guidelines. The highest probability of contamination should be estimated first to prioritize the risk assessment. The authorities need to be informed of the assessment's findings. If a potential contamination risk is identified, the holder of the marketing authorization should move on to step 2, as detailed below.[30]

Step 2- Confirmatory Testing

Confirmation testing should start as soon as the risk has been evaluated. Analytical testing for nitrosamine impurities should be performed as soon as possible on a high-risk product using sufficient sensitivity and validated methods. Similarly, within three years of the notification's publication, a confirmatory test of the entire medication in question needs to be conducted or otherwise justified. Regardless of the quantity found, nitrosamines must be reported to the appropriate authorities right away.[30]

Step 3- Changes to the Marketing Authorization

Any modifications to the drug product specifications or the manufacturing process of the active pharmaceutical ingredient that affects the marketing authorization must be made quickly. Public health authorities need to be notified right away if there is a risk. Primarily all stages need to be finished in three years.[30]

REGULATORY ASPECTS OF IMPURITY PROFILING

The efficacy, safety, and quality of pharmaceutical products are crucial factors in drug therapy. A drug's pharmacological and toxicological profile, along with any adverse effects brought on by contaminants in the mass and dosage form, all contribute to its overall safety. Drug impurities frequently have harmful pharmacological and toxicological effects that offset the therapeutic benefits of the drug. Medicines that are contaminated have side effects and are not safe, effective, or of high quality. Therefore, patient safety must be considered when releasing medications that fulfill the necessary quality standards onto the market. Therefore, it is crucial and required to provide the appropriate regulatory authorities with impurity data related to the isolation, identification, quantification, and control of impurities.[31]

LESSONS LEARNT

Investigations into nitrosamine contamination in sartans and other APIs have raised concerns about the suitability and effectiveness of the European Network guidelines, which were created in cooperation with foreign partners. These guidelines include ICH Q11, ICH Q3A (R2), and ICH M7.

The inclusion of more specific information in the regulatory documentation is necessary due to the significance of potential side reactions that could result in the formation of nitrosamines as well as careful consideration of the potential interactions between all materials and degradable substances.
The requirement for an open flow of technical information during process development, as well as later in the product life cycle, though better quality audits and improved quality agreements between manufacturers of active ingredients, manufacturers of third-party products, supplier qualification, and responsible parties. Concerns about confidentiality among holders of the marketing authorization, the Active Substance Master File (ASMF), and the Certificate of Suitability (CEP) for an active substance can seriously impede the necessary scientific evaluation.
The right change category was not always chosen since the requirements were not clearly understood.[22]

RECENT RECOMMENDATIONS TO INDUSTRY

In July 2020, the CHMP report on "Nitrosamine Impurities" which evaluates article 5(3), was released. It offers suggestions to producers on how to keep nitrosamines out of human medicine. The US Food and Drug Administration (FDA) released guidelines for the management of nitrosamine contaminants in pharmaceuticals intended for human consumption at the same time. The active ingredient and pharmaceutical manufacturers should conduct a risk assessment of their approved or marketed products as well as products with pending applications, according to the CHMP assessment report and FDA guidance, and then take the necessary steps to prevent or reduce the presence of nitrosamines in their goods.
To safeguard patients, the EMA also demanded in October 2020 that all organizations in charge of metformin-containing medications test their goods before releasing them onto the market.
For applications that are still pending, both authorities anticipate that the industry will take these corrective actions and they also demand that the applicant conduct the proper risk assessments and confirmatory testing (if required) throughout the assessment process.[22]

FUTURE PERSPECTIVES

Several precautionary steps have been suggested to anticipate and control suspected nitrosamine contamination or potentially mutagenic contaminants in medications. It is impossible to look into and analyze every conceivable danger using standard risk assessment hazards brought on by several odd chemical reactions that inadvertently occur during the production of pharmaceutical ingredients and finished products that may result in contaminants.[32]
Thirty-three of the pharmaceuticals under investigation showed suspected signals at the exact masses of their corresponding N-nitroso compounds (NOCs) linked to drug-nitrite interaction after nitrosation. The NAP test is now the first prospective method in drug development to identify and screen the nitrosation possibility on a target drug substance with all relevant reagents in synthetic reactions.[33]
As such, it is advised that NAP be used at the drug development stage and that it be included in the registration dossier for drug compounds and products. The second method to construct a more trustworthy control limit that is a crucial component of the risk assessment scenario is to use the estimated AIs based on the structure-activity relationships (SARs) of the current LD_{50} in the LCDB database.[32,34]

CONCLUSION

N-nitrosamine may pose a health risk when present in medications. When humans are exposed to certain N-nitrosamines over what is allowed and for an extended length of time, it can raise their risk of developing cancer. The presence of nitrosamine impurities in drugs can cause supply chain disruptions and even product recalls and withdrawals that result in bottlenecks. Regulators of the industry encounter difficulties in nitrosamine detection.
The addition of Nitrosamine Drug Substance Drug Related Impurities (NDSRIs) further complicates the matter.
Due to recent findings that N-nitrosamine types may have detrimental effects on human health, regulatory bodies, and pharmaceutical companies are working tirelessly to identify the underlying causes, how N-nitrosamines form, and the risks associated with N-nitrosamines. This is done to protect human health and take the necessary precautions to reduce or eliminate the presence of N-nitrosamines in active pharmaceutical ingredients (API) and medications.
Technologies like liquid chromatography, mass spectrometry, and gas chromatography are used to detect nitrosamine. These techniques aid in maintaining a low concentration of nitrosamine contaminants in a drug or drug product intended for use in human medicine.
Studies on nitrosamine have shown that a thorough evaluation of the possibility of nitrosamine contamination in a pharmaceutical product ought to take into account factors other than the concurrent use of nitrite and amine sources in the manufacturing process. All finished product manufacturers are required to evaluate and reduce these risks in any situation where nitrosamine content is a factor. Manufacturers can find a comprehensive list of considerations from the FDA and the European Union.
Nitrosamine levels below transient thresholds are deemed safe, allowing these products to stay on the market. The agency will be notified if nitrosamine levels are discovered to be above the LOQ value but within the AI limits. The applicant will then collaborate with the agency to address these concerns during review or if the agency thinks it's essential, right away before post-approval distribution. The marketing of these medications shouldn't in theory, be permitted in cases where the concentrations of nitrosamines are higher than permitted or when more than one nitrosamine is found.

To counteract the impact on the market, each national authority would evaluate the risk-benefit ratio if the product was taken off the market. By doing so, you will be able to assess the clinical effects of stopping or switching to a different therapy as well as decide whether or not other brands or therapies will be offered on the market.
To determine a viable control plan and an acceptable contamination threshold, as well as to assess the possible root cause of nitrosamine contamination, an approach based on risk assessment is advised. That being said, the risk assessment is predicated on the risk likelihood, which necessitates prior familiarity with event reports. The dangers will be disregarded or given little weight if there have been no prior threats, making the risk appraisal useless. As a result, the control methods for the preventative and corrective measures rely on the appropriate built-in notion of control limit criteria in addition to a thorough chemical understanding of the synthesis and decomposition of nitrosamines.

ABBREVIATIONS

FDA	Food and Drug Administration
NDSRIs	Nitrosamine Drug Substance Related Impurities
NDIPA	N-Nitroso diisopropylamine
NIPEA	N-Nitroso isopropylamine
NDBA	N-Nitroso dibutylamine
ICH	International Council of Harmonisation

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