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An Overview of Nitrosamine Impurities: Formation and Mitigation Strategies

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ABSTRACT

Nitrosamines impurities even in small amount known to be mutagenic and carcinogenic. Numerous recalls and enhanced regulatory scrutiny of drug substance and high-profile drug products batches contaminated with Nitrosamines have recently been carried out by several national authorities including FDA, EU, Canada, Australia, Switzerland, and Singapore. The pharmaceutical industry has witnessed recalls of several drugs, including those used to treat elevated blood pressure (including valsartan, losartan and irbesartan), heartburn and acid reflux (including ranitidine and nizatidine) resulting in heavy loss of revenue for the manufacturers and the temporary withdrawal of treatment for many patients worldwide. Regarding the control of these contaminants with the interim limit, the various regulatory authorities have released a press release or notice. It is imperative that drug product manufacturers identify and understand the probable source of nitrosamine formation in their manufacturing process and apply use mitigation strategies with proper controls to reduce the formation of Nitrosamines carcinogenic impurities. This review covers a variety of aspects that are essential to successful control measures against current and upcoming nitrosamine issues. as the accumulated knowledge of toxicity concerns and potential root causes of nitrosamine contamination, its accurate risk assessment, and various mitigation strategies have been explained in depth.

Keywords: Nitrosamine, carcinogenicity, varenicline, ascorbic acid.

1. INTRODUCTION

A large class of N-nitroso compounds (NOC) with a common functional >N-N=O group are referred to as nitrosamines1. Nitrosamines are a type of mutagenic and carcinogenic pollutants that are produced when secondary amines, amides, carbamates, compounds of urea with nitrite, and other nitrogen-containing agents react with nitrogen in the N(III) state. In some animal species, nitrosamine compounds are potent genotoxic agents, and the International Agency for Research on Cancer (IARC) has categorized some of them as 2A-likely or suspected human carcinogens [2]. The ICH industry advice M7 (R1) refers to them as "cohort of

concern" chemicals[3]. The discovery of nitrosamine impurities in drug substances and drug products has triggered major efforts by regulators as well as industries to reduce nullify or sometimes eliminate their presence in the drug supply chains. Through the employment of reagents, catalysts, solvents, or raw materials during the production process, these impurities may form and be integrated into drug substances or drug products. The food we eat, the water we drink, and even the air we breathe all contain nitrosamines [4] but the drugs that contain such impurities only add a little to a basal exposure which we all already have. To identify and quantify these contaminants using validated analytical procedures, it is necessary to use highly sensitive equipment that can detect these impurities down to trace level [5]. Also, the challenge in front of industries and regulators is how to prevent their formation in the formulation and choose the excipients that have no or very low nitrate or additional excipients that will not allow the formation of Nitrosamines in the formulation. How to assess and mitigate the risk is an ongoing exploration by the scientists.

Since 2018, many batches of pharmaceutical medicines have been taken off the market because of nitrosamine contaminants in excess of what is considered acceptable [6]. It was found that a number of pharmaceutical drugs such as ranitidine, nizatidine, metformin, and angiotensin-II-receptor antagonists (ARB) like irbesartan, losartan, and valsartan contain unsafe amounts of cancer-causing and mutation-causing contaminants of N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA)4. This news prompted pharmaceutical distributors all around the world to voluntarily recall many lots of these generic versions of ARB products[7]. FDA requested that all ranitidine medications be pulled off the market in the United States as of April 1, 2020, after learning in September 2019 that several popular products for heartburn (ranitidine, in products like Zantac, and nizatidine) were unsafe. Additionally, testing by the FDA in May 2020 revealed that some batches of extended-release metformin formulation had NDMA levels above the Agency's suggested tolerable consumption limit compelling the FDA to ask the named applicants to voluntarily recall certain batches of the said product and FDA advised companies on the best course of action [6].

The following are some general and root causes of nitrosamine formation [4], [6].

- Use of sodium nitrite (NaNO2), secondary, tertiary, or quaternary amines, or other nitrosating agents under acidic circumstances.

- Contaminated raw materials used in the production of active pharmaceutical ingredients (API).

- Utilization of recycled materials, including catalysts, reagents, and solvents.

- Contamination is caused by cross-processes that use the same line.

- Starting materials, intermediates, and drug compound production methods.

- This might also happen while a finished product is being created or stored.
- Processes involving beginning materials, intermediaries, and drug substances: This could also occur while creating a final product or while it is being preserved.

- Use of packaging materials: Nitrocellulose found within the lidding foil and printing ink containing dimethylamine and diethylamine, or diethylamine can react to form Nitrosamines e.g., dimethylformamide DMF, N-nitrosodiethylamine NDEA.

- Nitrocellulose acts as a nitrosating agent for secondary amines, present in printing inks,

forming N-nitrosamines in lidding foil. Their formation was confirmed by the addition of printing ink containing dimethylamine and diethylamine, or diethylamine alone, to lidding foil containing nitrocellulose primer.

- During the heat-sealing blistering process, this nitrosamine can be transferred to the finished product through vaporization and condensation.

2. FORMATION OF N-NITROSAMINE FROM API WITH AMINES

When referring to nitrosamine, it means a set of molecules, that contains a nitroso group attached to an amine as shown in figure 1. When Secondary, tertiary, or quaternary amines, as well as nitrous acid, its nitrite salts, or nitrocellulose, interact in an acidic environment, they form nitrosamine[4].

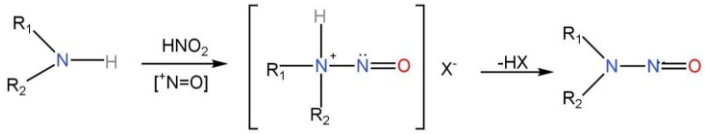


Figure 1: Representative reaction to form nitrosamines

Nitrosating substances add the N=O group to a molecule of an organic compound. Ex. Nitrous oxide (NO), tert-butyl nitrite (t-BuONO), dinitrogen tetroxide (N2O4), nitrosyl halides (XNO, X=Halogen), and nitrocellulose.

Nitrite is often first converted into nitrous acid for nitrosation to take place (pKa 3.37). This explains why acid is used to catalyze the nitrosation process. The nitrous acid is subsequently changed to a species that is actively nitrosating, such as nitrous anhydride (N2O3). The ratio of [amine] to [N2O3]2 determines the rate of nitrosation[8].

When nitrates and nitrites combine with secondary and tertiary amines, N-nitroso compounds (NOCs) are created [9].NOC is produced by drugs having a variety of nitrosatable groups, including N-alkyl amides, N-arylamines, hydrazines, cyclic secondary amines, dialkyl, alkylaryl, diaryl, and tertiary amines. Many APIs have secondary or tertiary amine functionality and are contaminated with nitrite or nitrate, which can trigger a nitrosation reaction (from excipients or packaging). A nitrosating agent is thought to be an electrophilic nitrosonium ion carrier [NO+] that is prone to attack by nucleophiles like amino compounds 10. Figure 2 shows the formation of NDMA from nitrite and dimethylamine [11]

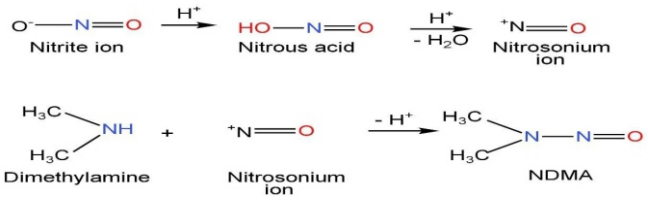
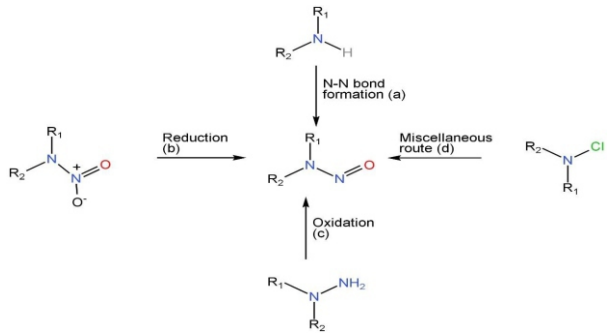


Figure 2: NDMA formation from nitrite and dimethylamine

The yield of NOC varies greatly depending on the reaction parameters, including the molar ratio of the drug to the nitrite, temperature, pH, and reaction time, in addition to the chemical components of the drug [8].

The most common way to make NOCs is through the production of N-N bond, which requires the use of electrophilic nitroso sources and NH-containing substrates such as secondary amines. Metal amides reacting with NO(g) create an alternate route (Figure 3, path a). Other, less-used techniques include oxidation and reduction processes using N-nitro derivatives and substances of the hydrazine- and hydrazone-types, respectively (Figure 3, paths b and c). Finally, a number of "miscellaneous" processes can lead to large quantities of N-nitroso compounds (Figure 3, path d)



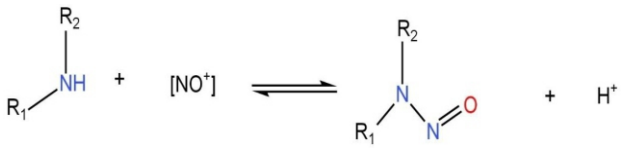
According to the following equations, the majority of

secondary amines are nitrosated8.

$$2HNO2 \leftrightharpoons N2O3 + H2O$$

$$R2NH + N2O3 \leftrightharpoons R2N.NO + HNO2$$

NOCs are typically made by N-nitrosating the corresponding NH-containing compound like secondary amine as a result of a nitrosating agent reaction, forming an N-N bond in the process. A nitrosating agent may be thought of as an electrophilic carrier of nitrosonium ion [NO+] that is attacked by a nucleophilic amino compound (Figure 4)[10], [13].



Most nitrosating compounds react with secondary amines at various rates, based on physicochemical or structural features such as their pKa and steric conditions around nitrogen. It has also been demonstrated that N-nitrosation occurs in several secondary NH-containing systems. Any molecule containing NH has the potential to go through electrophilic N-nitrosation, including amides, ureas, carbamates, guanidines, hydrazines, hydrazones, hydroxylamine ethers, heteroamides, and hydroxyl amines in decreasing order of reporting frequency [14], [15]. Because the NH moiety of these compounds has a weaker nucleophilic character than secondary amines, they are often less common and have lower reactivity. The exceptions are substances of the hydroxylamine and hydrazine types because they are often nucleus-loving.

Normally, stable nitrosamines are not produced by primary amines, but primary amines readily degrade to arenediazonium salts and deamination products (e.g., alcohols, ethers, alkenes, and so on), for aromatic and aliphatic amines respectively [10]. Although primary amines and nitrite react quickly, the presence of nearby hydrogens makes it possible for the nitrosamine species to change into a diazonium salt very quickly. Therefore, when just primary amines are present, the development of N-nitrosamine impurities is not seen as a significant danger.

It is also known that tertiary amines and their ammonium salts can directly undergo a dealkylative reaction with nitrites to produce N-nitrosamines. Trimethylammonium chloride and tetramethylammonium chloride can both produce N-nitrosamines almost at the same rate as quaternary ammonium salts through a related dealkylation reaction. These results have been attributed to nitrosative-style dealkylation processes[16]. Only tertiary amines with protons aligned with the nitrogen are suitable substrates for N-nitrosation[17]-[19]. It has been reported that tertiary amines are more than 1000 times less reactive than secondary amines because they need an additional dealkylation phase, with the breakage of the -CH bond being the rate-determining step [20].

3. ACCEPTABLE INTAKE LIMITS IN DRUG PRODUCTS

Based on Maximum Daily Intake, regulatory bodies have established temporary limits. The following acceptable intake (AI) levels are suggested by the FDA for the nitrosamine impurities NMPA, NIPEA, NDMA, NDEA, NMBA, and NDIPA Table 1. Manufacturers utilize these AI levels to establish nitrosamine impurity limits for APIs and pharmaceutical products 4, 22-24.

These restrictions only apply if a completed product has one N-nitrosamine. The maximum daily dose (MDD) of a drug, which is indicated on the drug label, is used to convert the AI limit into ppm (ppm = AI (ng)/MDD (mg)) [4], [22]-[24].

Table 1: NDMA, NMPA, NIPEA, NDEA, NMBA, and NDIPA AI limits in Drug Product

Nitrosamine		AI Limit (ng/day)
NDMA	N-Nitroso dimethylamine	96
NDEA	N-Nitrosodiethylamine	26.5

NMBA	N-Nitroso-N-methyl-4-aminobutyric acid	96
NMPA	N-Nitroso-N-methylaniline	26.5
NIPEA	N-Nitrosoethylisopropylamine	26.5
NDIPA	N-Nitrosodiisopropylamine	26.5

4. TESTING OF NITROSAMINE IMPURITY

Pharmaceutical companies must critically investigate the accurate identification, quantification, and monitoring of nitrosamine impurity levels. To detect low levels of impurities, the highest levels of selectivity and sensitivity of the analytical methods like HPLC-MS/MS and GC MSMS, are needed to ensure that manufacturers can verify the quality of the final products before they enter the market. The FDA and the EMA have both issued guidelines for the detection levels of angiotensin II receptor blocker's impurity based on daily dosage, but the limit of quantification has lowered to 0.03 ppm, making quantification even more difficult Table [24], [22-24]

Table 2: Nitrosamines Regulatory Guidelines .

Regulatory authorities	Testing		
	Routine testing	**Skip testing**	**Omission testing**
FDA	If a nitrosamine impurity is detected above the LoQ	No guidance provided	No guidance provided
EMA	LoQ should be ≤ of the acceptable limit based on AI	LoQ of the analytical procedure employed should be ≤ 30% of AI	LoQ of the analytical method employed should be ≤ 10% of the AI
ANVISA	Content > 10% of AI	No guidance provided	Admitted the absence of nitrosamines when <10% of the AI limit
SWISS MEDIC	Regardless of the quantity, if nitrosamines are found in APIs or pharmaceutical goods, companies must follow standard protocol,		
HEALTH CANADA	If the concentration of any nitrosamine is found to be at significant levels (e.g., greater than 30% of the acceptable intake) during confirmatory testing.	No guidance provided	No guidance provided

Chromatographic methods, such as reversed-phase liquid chromatography (RP-LC) or gas chromatography (GC), coupled with mass spectrometry (MS), spectrophotometry (UV), or nitrogen chemiluminescence, are some ways to test for nitrosamines in sartan (NCD).

EXCIPIENTS WITH NITROSAMINE IMPURITIES

Drug products may become unstable in the presence of reactive contaminants found in pharmaceutical excipients, which could lead to decreased product performance, potency loss, or the production of potentially dangerous degradants. Excipient reactive impurity levels may differ between suppliers and batches. To guarantee the effective development of pharmaceutical products, excipients should be extensively inspected for these contaminants early in the development of the formulation.

During the risk analysis of the drug product, additives are considered as a possible risk factor. Also taken into consideration are excipients with nitrites and weak amines. The most focus has been placed on nitrates and nitrites due to worries about nitrosating chemicals in excipients. Even though neither of these substances is a strong nitrosator on its own, it is conceivable that under specific circumstances they might interact with other chemicals to form nitrosamines. When the environment is only slightly acidic, nitrite creates the reactive molecule nitrous anhydride (N_2O_3) [21]. Enzymatic conversion of nitrates to nitrite might result in the reactive nitrous anhydride in acidic circumstances[25]

Nitrates and nitrites, two common nitrosating pollutants, are present in excipients at levels of a few parts per million. From 0.9 ppm in a sample of hydroxypropyl cellulose (HPC) to 285.6 ppm in a sample of sodium starch glycolate (SSG), nitrates were present in the excipients. Excipients such as lactose fast float, pre-gelatinized starch, croscarmellose sodium, sodium starch glycolate, povidone, and crospovidone

contain trace quantities of nitrate or nitrite impurities [26]. To screen for traces of nitrate and nitrite in excipients, an ion exchange chromatography method was developed employing an analytical column (AS-11 Dionex) and a guard column (AG-11 Dionex).

For biologics, where the final drug product is typically a solution formulation, amino acid excipients (like L-histidine, L-arginine, and L-proline) and other weak amine-containing excipients (like triethanolamine) have the potential to react with nitrosating agents and form nitrosamines within the drug product. Although amino acids might be nitrosated, these nitroso compounds have not been associated with cancer in the literature [27-29]. The potential risk that might arise from the presence of nitrite and the susceptible amine in an excipient in trace amounts will depend on the formulation composition, and it should be evaluated in accordance with that.

The drugs listed in Table 3 have been tested for formation of NOC the large majority of which have been found to form NOC or in some cases, other reactive species by reacting with nitrite.

Table 3: Example of drugs tested for the formation of NOC by reaction with nitrite

Drug	% NOC formation	Ref	Drug	% NOC formation	Ref	Drug	% NOC formation	Ref
Ajmaline	80%	30	Alprenolol	91%	31	Aminapyrine	87.50%	31
Bamethan	80%	30	Cimetidine	90%	32	Dilazep	94%	30
Dipyrone	100%	31	Metham-Phrtamine	94%	32	Procainamide	75-100%	33
Propranolol	94%	30	Ranitidine	89%	34	Trimetazidine	98%	30
Ranitidine	55-65%	35	Antipyrin	0 - 60.8%	36	Azapropazone	25-49%	33
Bromazepam	25%	37	Cefradine	30%	33	Chlordiaze Poxide	55%	38
Chloroquine	15%	33	Cinnarizine	42.80%	39	Clonidine	75%	30
Ephedrine	62-68%	33	Furosemide	50-52%	33	Allopurinol	0% pe	33
Ambroxol	<0.001%	40	Atenolol	0.005-0.012%	41	Benzathine	< 0.001%	40
Bromhexine	0-0.02%	42	Cephalo-Sporine C	0.68%	42	Metformin	<0.001-0.001%	35
Oxazepam	0%p	33	Piperazine	0.32%	40	Verapamil	0.1-1.8%	35
Ethambutol	0.26%	40	Piperazine	100%	31			

CASE STUDY OF VARENICLINE (CHANTIX™):

Varenicline (Chantix™) is a selective alpha4-beta2 neuronal partial agonist of the nicotinic acetylcholine receptor that aids in adult smoking cessation. initially approved by the FDA in 2006 and released to the market by the Pfizer business. In August 2008, it received SFDA permission for import. The active pharmaceutical component (API) in Chantix™ is varenicline tartrate. The drug is taken to help people stop smoking.

Since 2021, the FDA is informing patients and healthcare professionals that Pfizer is expanding its voluntary recall to include every batch of varenicline (Chantix™) 0.5 mg and 1 mg tablets. Because of potentially harmful N-nitroso-varenicline levels. In May 2022 FDA was confident that manufacturers will be able to administer varenicline to patients with N-nitroso-varenicline impurities at or below the agency's permitted daily consumption limit of 37 ng. The N-nitroso-varenicline impurity shall not exceed that limit in any freshly manufactured varenicline intended for the American market [43].

It has been noticed that N-nitroso varenicline may be present or developed in the medicinal product. To ensure the security and caliber of the varenicline tartrate drug product (Chantix™) and drug substance, the agency created and validated a method to determine the presence or absence of varenicline Nitroso-Drug Substance Related Impurity (NDSRI). The structure of the NDSRI varenicline is shown in Figure 5 below [44].

Figure 5: Varenicline Nitroso-Drug Substance-Related Impurity

For chantix™ drug product and Varenicline drug Substance Varenicline Nitroso-drug substance related Impurity (NDSRI) determination by liquid

Chromatography-High Resolution Mass Spectrometry (LC-ESI-HRMS) method was utilized.

The following processes are used to create the nitro reduction impurity and varenicline intermediate: via hydrogenating Pd/C, hydrogenating 2,3,4,5-tetrahydro-3-(trifluoroacetyl)-1,5-methano-1H-3-benazepine, and selectively reducing nitryl, a target chemical with formula is obtained (I). The process for making the varenicline intermediate and its nitro reduction impurity has the benefits of not polluting the environment, avoiding the need for column chromatography, and increasing product yield and purity[45].

5. RISK ASSESSMENT

(Nitrosating chemicals sources)

As nitrosating agents, nitrites (such as sodium nitrite, $NaNO_2$), nitrous acid (HNO_2), nitric oxide (NO), nitrosyl halides (such as ClNO, BrNO), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), and organic nitrites (such as t-BuONO) should be taken into consideration[46].

(Secondary and tertiary amines sources)

Triethylamine, diisopropylethylamine, and N-methylmorpholine are examples of tertiary amine bases that have been linked to the production of N-nitrosamine due to their ability to degrade to secondary amines. Amines may also be introduced to raw materials, intermediates, or the API itself as contaminants or degradants. Common amide-containing solvents include N, N-dimethylacetamide (DMAC), N, N-dimethylformamide (DMF), and N-methylpyrrolidinone (NMP). Primary amines include monoethylamine and quaternary ammonium salts include tetrabutylammonium bromide (TBAB)[47].

Modelling of reaction kinetics can be used to reduce the danger of the development of nitrosamines by estimating the worst-case reaction speed and comparing it to the environmental circumstances the product is exposed (pH, temperature, reaction duration). For aqueous solutions, the formulation pH is the most important variable, but other elements like heat, the sequence of addition, and formulation concentration all matter It is known that the pH range between 3 and 4 is optimal for nitrosation with nitrous acid, and that higher pH ranges significantly reduce the likelihood of the nitrosation reaction. If pH > 7 is achieved for both the process and the product, the danger of nitrosation of amines with trace nitrite is insignificant. There is some evidence that a pH range of 5 to 7 can also carry a low risk of nitrosation, but this must be taken into account on an individual basis [48]. Drug product risk assessment steps are mentioned in Figure 6[49]

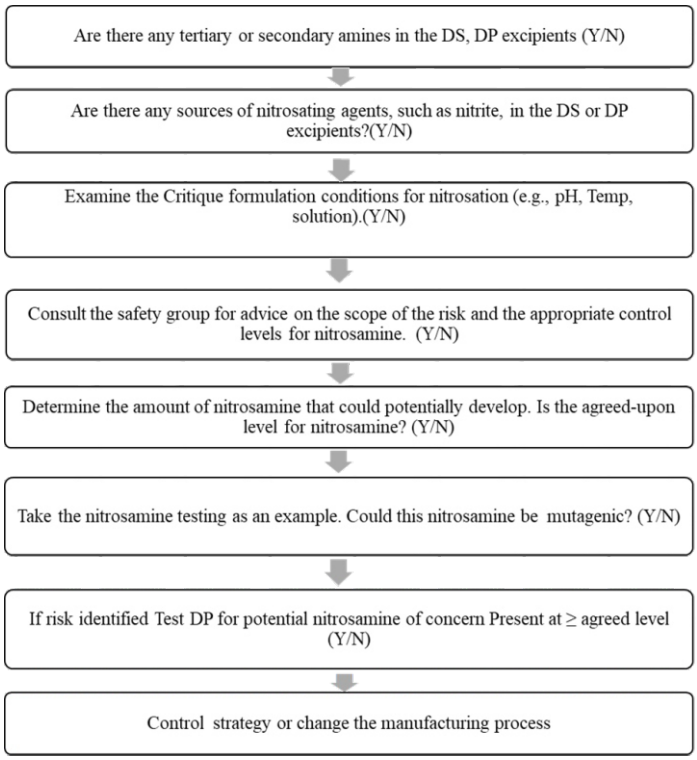


Figure 6: Drug product risk assessment

6. STRATEGIES FOR MITIGATING THE RISK OF NITROSAMINE

A safe and effective method of reducing the risk of impurities in drug products is to prevent the formation of nitrosamine. Formulation Designs that incorporate excipients such as sodium carbonate that modify the microenvironment to neutral or basic pH, will help to inhibit the formation of nitrosamine Ascorbic acid and alpha-tocopherol have numerous advantages when used as excipients in show formulation, including:

Effective nitrosamine inhibition (>80%) and also

antioxidants function as stabilizers in the formulation of finished pharmaceuticals [50].

Antioxidants like ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) suppress the development of nitrosamines in acidic conditions. while their action is slowed down in neutral or basic conditions. Formulation designs that use excipients like sodium carbonate that alter the microenvironment to a neutral or basic pH can inhibit NDSRI production [51]. When possible, the use of bases other than secondary, tertiary, or quaternary amines might reduce nitrosamine formation. The amide solvents such as N, N-dimethylacetamide, N-methylpyrrolidone, and N, N-dimethylformamide should be used with caution. Substituting various quenching agents for nitrites in the processes of azide decomposition. Whenever feasible, avoid reaction circumstances that might result in the production of nitrosamines. If this is not possible, show by suitable and thorough fate and purge tests that the process is appropriately regulated and capable of consistently lowering nitrosamine impurities.

6.1 NITROSATION INHIBITORS

The N-nitrosation process has been demonstrated to be inhibited by a wide range of synthetic or naturally occurring substances or combinations Table 4. NOC formation can be minimized or eliminated by the presence of totally preventing chemicals that quickly eliminate nitrosating substances or transform them into inert substances. A variety of factors affect how much N-nitrosation is inhibited, including (i) the relative and absolute concentrations of the nitrosating species, the inhibitor, and the amine as well as (ii) the nitrosating agent's respective rates of reaction with the inhibitor and with the amine. The amine that acts as the substrate for the nitrosating species is often competed for by such reagents. N-nitrosation is impacted by both pH and catalyst presence.

Table 4: Inhibitors of the formation of N-Nitroso compounds in vitro

Vitamins & and sulphur compounds	Phenolic compounds	Miscellaneous compounds	Unsaturated hydrocarbon
Ascorbic acid	Butylated hydroxytoluene	Sorbic acid	Squalene
α-tocopherol	Butylated hydroxy anisole	Urea	-
Cysteine	-	-	-
Methionine	-	-	-

6.2 ASCORBIC ACID

Ascorbic acid is known to react with a variety of nitrosating substances and is practically nontoxic to humans. Under anaerobic conditions, ascorbic acid can typically react with N2O3, H2NO2+, and NOX at rates that are higher than the corresponding nitrosation rates for dialkylamines or amides. Therefore, ascorbic acid can generally prevent these classes of compounds from nitrosating in vitro [52, 53]. This characteristic has been used to inhibit in vivo nitrosation and prevent nitrosamine formation in food[54]

Ascorbic acid as well as the ascorbate anion prevent the development of NOC in aqueous solutions across a pH range of 2 to 5 by quickly reducing nitrous acid to NO and producing dehydroascorbic acid figure 7. To prevent N-nitrosation when catalysts or air are present, ascorbic acid must be utilized in excess. It is water soluble, therefore Ascorbic acid is ineffective at preventing nitrosation in lipophilic media.

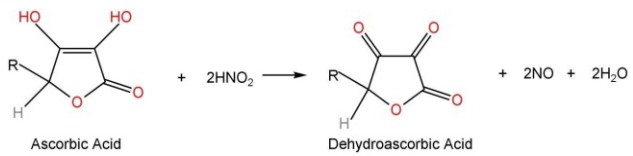


Figure 7: Ascorbic acid reducing Nitrous oxide to NO

For instance, the equilibria depicted in Figure 8 involve several nitrosating species that can combine with ascorbic acid to create NO, a molecule that does not nitrosate amines. These reactions may then deplete the system's ability to nitrosate under anaerobic conditions. However, oxygen can react with NO to produce the nitrosation-capable gases N2O3 and N2O4 (as shown in Figure 8)

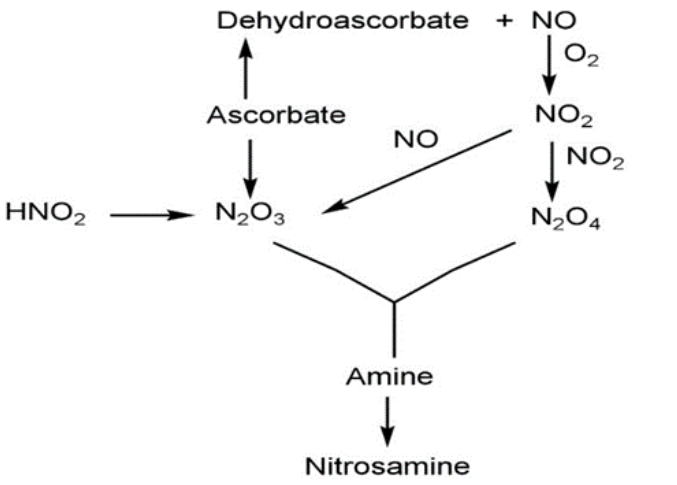


Figure 8: Method of Ascorbic acid forming nitrosamine

6.2.2. Advantages and disadvantages of using ascorbic acid to prevent nitrosamine formation.

Advantages

1) It is an approved pharmaceutical substance appearing in the FDA non-active pharmaceutical ingredient database, it is generally recognized as safe GRAS.
2) Pharmaceutical quality is described in the USP, B.P, and Ph. EU. So, it is a standardized material unlike some of the suggested scavengers based on polyphenolics found in plant extracts.
3) In the regulatory process it will be easily acceptable as a safe material and is already recognized for its use as an antioxidant in many formulations so there will be no regulatory barriers in terms of toxicology.
4) There may be added possibilities as there are preliminary reports that it may be more effective as a nitrosamine preventative when combined with either tocopherol or citric acid.

Disadvantages

1) The precise amounts of Ascorbic acid required to prevent nitrosamine formation will differ from product to product and may affect the overall manufacturing process.
2) Ascorbic acid is not chemically inert and may react with some drug substances".
3) The regulatory authorities will require new pharmaceutical data. Proof of effectiveness in preventing NA formation.
4) New stability data will almost certainly be required.
5) Bioavailability (general acceptance of biowaivers based on comparative dissolution data unlikely for NTI, MR, or BCS class 4 products)

6.3 α-TOCOPHEROLS

Phenolic substances, polyphenols, and tocopherols are all efficient inhibitors of N-nitrosation in lipids and emulsions in water because they diminish nitrite. a - Tocopherol esters, especially acetate, are only effective as N-nitrosation inhibitors after being hydrolyzed in vivo by lipase[55].

Depending on their structure and the conditions of the process, several straightforward phenols and phenolic compounds can slow the creation of NOC [56]. Phenolics typically react with nitrite in acidic circumstances more quickly than the majority of amino compounds. Catechol, hydroquinone, 1,2,3-dihydroxy phenols, and other 1,2- and 1,4-dihydroxy phenols, para-substituted phenolics, such as vanillin, and several naturally occurring polyphenols, such as pyrogallol and gallic acid, the tannins (phenolic, cinnamic, and chlorogenic acids) [57].

6.4 MISCELLANEOUS COMPOUNDS

Alcohols and carbohydrates easily react with an acidic aqueous nitrite solution or with nitrosyl chloride to produce nitrite esters (alkyl nitrites, RONO) [58]. Thus, it has been demonstrated that in acidic conditions, simple alcohols and carbohydrates partially limit the synthesis of N-nitrosamines from secondary amines and nitrite [59].

7. CONCLUSION

Nitrosamine impurities are considered highly potent mutagenic carcinogens and require strict control to minimize human exposure. The nitrosamine contaminated high profile drugs and drug product recalls had profound effects on loss of business, reputation, and trust for the pharmaceutical companies and for the regulatory agencies that oversee

their operations. To limit the risk of nitrosamine impurities in drugs, there was an imminent legal obligation in several markets for manufacturers to present reformulation strategies and mitigation plans by Q3 2022. The analysis of nitrosamines with validated methods conforming to GMP norms can be challenging with the quantification of ultra-low levels of these impurities in diverse and complex matrices. New analytical techniques and workflows could be of great advantage to detect and quantify these carcinogenic nitrosamine impurities along with batch-to-batch monitoring for comparison quickly and decisively. A potential mitigation strategy which is also a US FDA recommended approach is including antioxidants like ascorbic acid and alpha- tocopherol as nitrosamine blockers and to redesign the formulations. The use of high-quality excipients produced and governed by a wide range of global certifications including good manufacturing practice GMP, certification of suitability CEPs, drug master file DMFs, and complying with multiple pharmacopeias will help mitigate the risk of nitrosamine impurity formation in the drug products. Another strategy is to develop new drugs with processes and chemicals to minimize the risk of nitrosamine formation.

It is imperative to develop pharmaceutical products free from nitrosamine contaminants, ensuring adherence to regulatory standards.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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