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An Inclusive Insight on Nitrosamine: A Reminiscent Carcinogen

[1]Ms. Pranali B. Bhore*, [2]Dr. Priyanka Bhore Lamba, [3]Dr. Bhushan B. Bhore, [4]Dr. Rupesh Pingale

[1]NCRD's Sterling Institute of Pharmacy, Nerul

[2]Shamal Hospital and ICU Care, Belapur

[3]Shamal Hospital and ICU Care, Belapur

[4]NCRD's Sterling Institute of Pharmacy, Nerul

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ABSTRACT:
Nitrosamines, are the potent carcinogenic compounds and are having significant concern in pharmaceutical industry. Specifically, NDMA is a probable human carcinogen. Nitrosamines having the presence of a nitroso and amine group, reacted in the acidic pH and/or under high temperature conditions. Its presence even at trace quantities in medicines concerns due to their carcinogenic and genotoxic nature, confirmed by the animal studies. Hence regulatory authorities implemented stringent guidelines for prohibiting occurrence of these impurities in pharmaceuticals. These impurities may form during API synthesis, can come from the excipients, raw materials, and recovered solvents or it may be the result of degradation pathway. To prevent the occurrence of these impurities it is important to perform vendor qualification, establishing specification for impurities, proper selection of raw materials, reagents, solvents, and packaging material evaluation. This article reviews the history, chemistry, formation, sources, preventive strategies, and acceptable intake limit of nitrosamines.

History:

Nitrosamines are not newer to the industry, it is already known to leather, food processing (to as some of the nitrosating agents are used as preservatives that are precursors for the formation of nitrosamines), detergent, and dye industry. These industries knew that nitrosamines are carcinogenic in nature, but no one thought that these impurities may enter the pharma industry.

In June 2018, regulatory authority like, FDA identified the presence of N-nitrosodimethylamine (NDMA: one of the Nitrosamine impurity present into the drug substance or drug products) in Valsartan; with which the Nitrosamine impurities introduced in the world of pharmaceutical industry.

In September 2019, FDA got aware that along with the Valsartan, some chest pain products such as ranitidine (Zantac), and nizatidine (Axid) contained unacceptable levels of NDMA with which Nitrosamine era started.

In December 2019, FDA informed about the metformin; manufactured in some of the countries has NDMA in it. **[1]**

Introduction:

Nitrosamines, many of which are possible human carcinogens. The term Nitrosamine (R1-N-R2)-N=O is the class of compounds having the combination of amine group (secondary, tertiary, quaternary amine) and nitroso group (N=O). The amines and nitrosating agents react together under acidic condition to form nitrosamines. Examples of nitrosating agents are sodium nitrite and tert butyl nitrite (which are nitrites), nitrous acid, nitrite oxide, nitrosyl halides, dinitrogen trioxide, dinitrogen tetroxide. Controlling nitrosating agent is a

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very difficult task, its content at part per billion level can also forms the nitrosamines when it meets amines. **[2]**

Potential control strategy to avoid formation of nitrosamines:

Use of primary amines in the reaction.

Why not primary amines forms nitrosamines?

After reacting with nitrosating agents' primary amines forms unstable and highly reactive diazonium ions, which further decompose to molecular nitrogen. Hence, we can avoid the formation of nitrosamine impurities by using primary amines over the secondary, tertiary, or quaternary amine.

Based on animal studies, it was found that nitrosamines are class of chemical compounds which are human carcinogens. Nitrosamines can also be the result of degradation; Example: degradation of ranitidine produces the NDMA impurity. Active Pharmaceutical Ingredients in which these impurities commonly detected are ranitidine, nizatidine, metformin, valsartan, losartan, pioglitazone, olmesartan, and irbesartan.

Valsartan and losartan are badly impacted compounds, why?

Because in sartan, during the formation of tetrazole ring if solvent like dimethylformamide (DMF) used then certainly nitrosamines get formed in these compounds.

ICH M7 guidelines, refers nitrosamine impurities as Cohort of Concern. These guidelines deal with the mutagenic impurities and when these impurities limit exceed beyond the threshold of toxicological concern (TTC) then that compound is known as Cohort of Concern and nitrosamine is one of it.

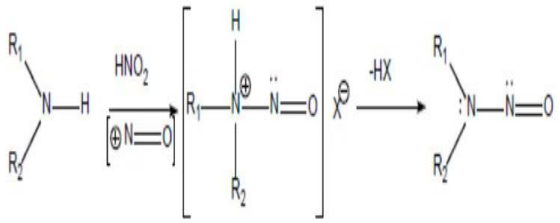

Figure 1: Representative reaction to form nitrosamines [3]

Nitrosamines identified by FDA:

Seven different types of nitrosamines identified till now by Food and Drug Administration (FDA) as well as, European Medicines Agency (EMA), Brazilian National Health Surveillance Agency (ANVISA), Swiss authority (Swissmedic), Health Canada.

Identified nitrosamines are as shown in figure below:

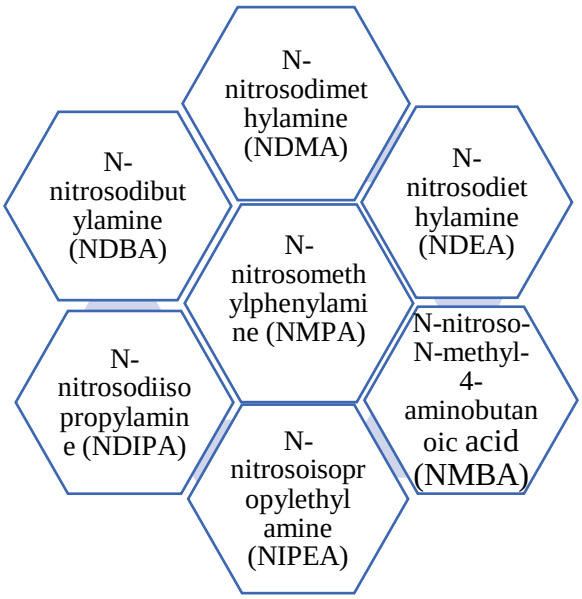

Figure 2: Identified nitrosamines by FDA [4]

Nitrosamine drug substance related impurities (NDSRI's):

NDSRI's is the class of nitrosamine which is Fastly growing and most dangerous. NDSRI's shares structural similarity to API. Commonly used excipients and water is the root cause for the formation of NDSRI's. **[5]**

Sources of nitrosamine impurities:

1. General conditions that lead to the formation of nitrosamine impurities:

a. The reaction of nitrite salts and amines (specifically secondary, tertiary, quaternary) under acidic conditions form the nitrosamine.

b. By addition of amines and nitrosating agents into different steps cannot confirms the absence of formation of nitrosamines. When one step uses nitrites as reagent, its carry over into the next step and subsequent

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reaction with amines can generate the nitrosamine impurities.

How to control:

i. By performing carryover testing of amines and nitrosating agents
ii. By developing and validating analytical method for amines and nitrosating agents as well setting specification levels for them

2. Sources such as starting materials with Secondary, Tertiary, Quaternary amines form nitrosamines:

a. API or starting material or intermediate with amine (secondary or tertiary) functional group in presence of nitrosating agents form nitrosamine. Example: Ranitidine, itself has secondary amine in it; which further may form nitrosamines

b. Amine solvents (e.g. DMF, N, N-Diethylacetamide) under high temperature, forms secondary amines. DMF at higher temperature yields dimethylamine which is secondary amines may further react with nitrous acid to form NDMA.

How to control:

i. Using primary amines
ii. Avoid usage of amine solvents like DMF, instead use benzyl alcohol, isopropyl alcohol
iii. Avoid high temperature during the reaction when using DMF as solvent in the reaction which further prevents formation of dimethyl amine (secondary amine)

c. Use of nitrosating agents such as sodium nitrite ($NaNO_2$)

How to control:

i. Avoid the acidic condition
ii. Maintain the time of the reaction

d. The reagents or catalyst in the form of quaternary amines can react with nitrous acid to form nitrosamines.

How to control:

i. Use of Basic condition during reaction
ii. Use primary amines as catalyst instead of secondary amines
iii. Maintain reaction temperature at low. Avoid high temperature during reaction

e. Secondary amines present as impurities in amide solvents.

How to control:

i. Set the specifications for reagents with respect to amines. For example, if using solvent like DMF then set the specification level for dimethylamine. As the presence of dimethylamine, one of the impurity present into solvent DMF is a secondary amine which further reacts with nitrosating agent to form nitrosamine impurity
ii. Vendor qualification for the solvents used during the reaction
iii. Use alkaline pH during the reaction

3. Contaminated raw materials from the vendor itself:

a. Use of contaminated starting materials and intermediates by nitrosamine.

How to control:

i. Set specifications for raw materials
ii. Performing vendor qualification
iii. Take risk assessment and declaration from vendor

b. Cross-contaminations due to manufacturing into same area.

How to control:

i. Critical vendor qualification needed

c. Starting material such as sodium azide having the known impurity, sodium nitrite which react with amines under acidic conditions to form nitrosamines.

How to control:

i. Set specification levels for raw materials with respect to nitrosamine impurities
ii. Vendor qualification

d. Some of the raw materials having secondary or tertiary amine as an impurity

How to control:

i. Set specification levels for raw materials with respect to nitrosamine impurities
ii. Vendor qualification

4. Source of contamination:

a. Recovered materials such as solvents, reagents, and catalysts from different steps and its subsequent use may results in the formation of nitrosamine impurities due to the presence of residual amines.

Journal of Chemical Health Risks

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How to control:

i. Set specification of raw materials
ii. Proper storage of solvents
iii. Control during distillation

b. The comingled solvents from different processes can introduce nitrosamine impurities.

How to control:

i. Avoid blending from different sources
ii. Purification before use
iii. Control during distillation

c. If recovery step given to third party contractor (e.g., solvents, reagents, and catalysts recovery).

How to control:

i. Vendor qualification
ii. Set specifications
iii. Maintain purification process data same as that of batch manufacturing records
iv. Maintain the reagent and catalyst quality as per material specifications
v. Prepare the common method for detection of nitrosamines impurities

5. Inadequate cleaning of equipment between different processes or product formation can cause raw material contamination.

6. Heat as the Source of Nitrosamine Contamination:

a. If the recovery process involves heating of nitrous acid, nitrosamines could form during solvent recovery.

How to control:

i. Use of another reagent

7. Inadequate knowledge of process and Control:

a. Lack knowledge of the manufacturing process for APIs. For example, lack knowledge about process optimization like specific temperature, pH, or the sequence of adding reagents, intermediates, or solvents

8. Nitrosamine impurities found in Drug products (apart from raw materials):

a. Use of excipient containing Nitrite impurities may lead to nitrosamine impurities
b. Drug product which forms nitrosamine impurity during shelf life

9. Nitrosamine formed during analysis (GC analysis)

How to control:

i. Use another analytical technique for example, LC-MS/MS

Strategies to control Nitrosamine formation:

1. Conduct Supplier Qualification

a. Check for potential nitrite impurities across excipient/raw materials/API
b. Check for potential amine impurities across excipient/raw material/API
c. Check for potential Nitrosamine cross contamination in sourced material
d. Set specification for content of amines/nitrosating agent/nitrosamines in sourced material

2. Antioxidants

a. Vitamin C & vitamin E: inhibit the formation of NDSRI's

Examples of NDSRI's:
N-Nitroso irbesartan
N-Nitroso Vernicline
N-Nitroso-Phenyl hydrazine

3. Maintain Basic or Neutral Environmnet

a. Basic or neutral environment reduces the risk of formation of nitrosamines significantly

4. Avoid carryover of Nitrosating agent /amines to next step

5. Avoid elevated temperatures

a. When DMF heated at elevated temperature then it converted into Dimethylamine which is secondary amine. Further if nitrosating agents' presence may develop nitrosamine impurity.

6. Avoid amines/nitrosating agent as an impurities as some excipients contains secondary amines as an impurity

Examples:

a. Triethylamine contains dipropylamine and isopropylethylamine
b. DMF contain Dimethylamine
c. Sodium nitrite is known impurity in sodium azide
d. potassium nitrate contains nitrite impurity

7. Avoid comingling of recovered solvents: It is the common practice in synthesis of APIs to use recovered solvents. But comingling of solvents may lead high risk of formation of nitrosamines.

Journal of Chemical Health Risks

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8. Use validated/controlled process for recovery of solvent reagents, catalyst at 3rd party
9. Use of primary amines

a. Use primary amines rather than other amines

10. Avoid Nitrocellulose laminated blister

a. nitrocellulose printing primer and amines from printing ink react to generate nitrosamines. **[6]**

Acceptable intake limits of nitrosamines in drug products (DP) as per FDA guidelines:

Below mentioned six impurities are found to be nitrosamines identified by FDA. Each impurity with its Acceptable intake (AI) given in nanogram/day (ng/day).

Table 1: Acceptable intake limits if only one nitrosamine impurity present

Name of the impurity	Al (ng/day)	ppm limit if MDD is 880mg/day
NDMA	96	0.11
NDEA	26.5	0.03
NMBA	96	0.11
NMPA	26.5	0.03
NIPEA	26.5	0.03
NDIPA	26.5	0.03

1. The Al limit is defined as the daily exposure to a compound

For example, NDMA has the 1:100,000 cancer risk after 70 years of exposure.

2. Al limit to part per million (ppm) conversion calculated by drug's maximum daily dose (MDD)

ppm = Al (ng) / MDD (mg)

1. Above limits are only for single nitrosamine impurity in DP
2. If more than one of the nitrosamine impurities present

a. MDD of less than 880mg/day:

Total nitrosamines: **Limit: NMT 0.03 ppm**

b. MDD of more than 880mg/day:

Total nitrosamines: **To be adjusted such that Al does not exceed 26.5ng/day**

Example: MDD = 1200mg

Hence the formula for calculating ppm = Al (ng)/MDD

ppm = 26.5/1200

ppm = 0.02

Limit: NMT 0.02ppm. **[7]**

Conclusion:

Identification of nitrosamines in pharmaceutical products having utmost importance as trace quantities also cause potential health risks. The review article outlines and discusses potential sources and effective control strategies. Raw materials, reagents, manufacturing processes, degradation pathways, and storage conditions are the major sources of impurities. Understanding these sources are essential to prevent their occurrence which further ensures drug safety.

Strategies to control nitrosamine impurities are: risk assessments, careful selection of raw materials, optimization of synthetic routes, control over packaging and storage. Further management of nitrosamine impurities ensures delivering effective, high-quality medicines worldwide.

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