

Nitrosamine Impurities in Pharmaceuticals: Root Cause Analysis and Mitigation Strategies for Valsartan, Metformin, and Rifampicin

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Abstract

Nitrosamine impurities are genotoxic and carcinogenic, and have emerged as a big quality and patient safety concern in the pharmaceutical industry. Their formation could happen during storage or production of pharmaceuticals, as a result of interactions between precursors containing amine and nitrosating agents. In this review, the formation pathways, sources and mitigation strategies for nitrosamine impurities in three selected pharmaceutical products (valsartan, metformin, and rifampicin) are compared. The chemical mechanism of nitrosamines formation is explained and a drug specific root cause analysis (RCA) is done using Five-Why analysis for valsartan, Fishbone (Ishikawa) analysis for metformin and Fault Tree Analysis (FTA) for rifampicin. The production conditions for use of sodium azide, sodium nitrite, and dimethylamine sources were correlated with the formation of N-nitrosodimethylamine (NDMA) associated with valsartan. For metformin, there was a correlation between the presence of NDMA, the presence of dimethylamine as a trace API impurity, atmospheric nitrogen dioxide and granulation conditions. A 1-methyl-4-nitrosopiperazine, MNP, was found to be formed in rifampicin, below conditions were responsible for the MNP formation: 1-amino-4-methylpiperazine degradation, oxidative conditions, storage related factors. Evidence-based mitigation strategies like Process Optimisation, Precursor Control, Material Control, Validated Analytical Testing, Batch-release control and Continuous Monitoring are also evaluated. Finally, the review demonstrated that a science- and risk-based approach that incorporates a drug-specific strategy, including chemical understanding and structured RCA, as well as the regulatory assessment and appropriate corrective and preventive measures is required for control of nitrosamines.

Keywords: Nitrosamine impurities; NDMA; MNP; valsartan; metformin; rifampicin; root cause analysis; Five-Why; Fishbone analysis; Fault Tree Analysis; risk assessment; mitigation strategies.

1.Introduction

Nitrosamine impurities have become a significant pharmaceutical quality and patient safety issue due to their high genotoxicity and carcinogenicity. Compounds containing nitroso groups attached to an amine may be formed when certain amine containing compounds react with nitrosating agents under appropriate chemical conditions [1]. There are several nitrosamines that are classified as probable or possible human carcinogens and are considered to be important mutagenic impurities that must be controlled, such as N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and N-nitroso-N-methyl-4-aminobutyric acid (NMBA) [2].

It was made more apparent after the discovery of NDMA in valsartan-containing medications in 2018, leading to recalls and thorough regulatory investigations. Later, nitrosamine impurities were found in several therapeutic classes, such as angiotensin II receptor blockers, histamine-2 receptor antagonists, antidiabetic drugs and antimicrobials like rifampicin and rifapentine. These contamination events have illustrated that nitrosamine contamination can be derived from a variety of sources: starting materials, solvents, reagents, manufacturing/process, excipients, environmental, equipment and degradation during storage [2].

Nitrosamine formation generally involves nitrosatable amine precursors and nitrosating agents like nitrous acid, nitrite, dinitrogen trioxide or dinitrogen tetroxide. This is especially true for secondary amines as they can be directly nitrosated to give stable nitrosamines and tertiary amines can be dealkylated to form secondary amines which can then be nitrosated. The amount and type of nitrosamine formed are dependent on pH, temperature, reaction time, solvents, presence of nitrosating agents, and presence of susceptible amine precursors [2].

Root cause analysis (RCA) in pharmaceutical manufacturing has been identified as a critical issue with regards to nitrosamine impurities. It is important to determine the exact source of a nitrosamine as the factors that may contribute to the formation of nitrosamines in a drug substance and in a product are often different. In the case of valsartan, changes to the API manufacturing process (such as the use of sodium azide, sodium nitrite quenching, and dimethylformamide-associated dimethylamine) were found to be associated with the formation of NDMA; in the case of metformin, dimethylamine, nitrosating conditions, and manufacturing/environmental factors were found to be associated with NDMA formation; in the case of rifampicin, an amine precursor (from the API) and possible degradation/oxidative pathways were found to be associated with the formation of 1-methyl-4-nitrosopiperazine (MNP).

To address these concerns, regulators have developed measures to mitigate and manage nitrosamine impurities through risk assessment. The U.S. Food and Drug Administration (FDA) advises three-step process: 1) risk assessment; 2) confirmatory testing if a risk is identified; and 3) implementation/reporting of appropriate measures to minimize and/or eliminate nitrosamine impurities. The recently issued FDA guidance further breaks down the distinction between small molecule nitrosamines and nitrosamine drug substance related impurities (NDSRIs), highlighting the need to assess vulnerable APIs, manufacturing processes, excipients, and other potential sources during the product lifecycle[1].

As such, a systematic knowledge of the chemical pathways, the root causes of the contamination related to the drugs and the evidence-based mitigation strategies is critical for the effective control of nitrosamine contamination. This review highlights the chemistry of the nitrosamine formation and uses the appropriate RCA methodology to three representative pharmaceutical studies in order to determine the different causal pathways associated with these products. The review also outlines the key contributors and assesses the evidence-based corrective and preventative actions such as process change, control of nitrosamine precursors and reagents, analytical testing, and continued monitoring, and offers an integrated approach to prevent and control nitrosamine impurities in pharmaceutical products.

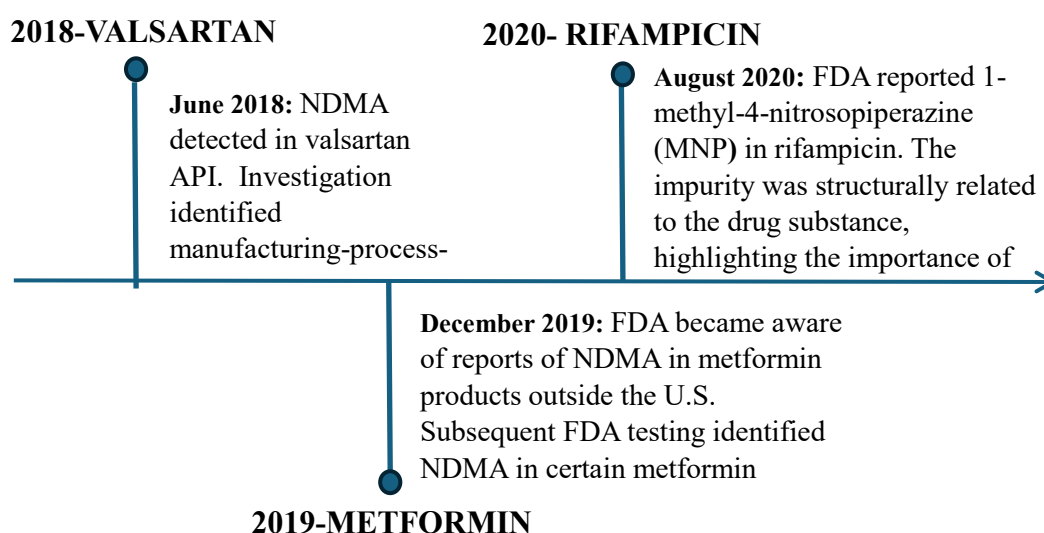


Figure 1 Timeline of reported nitrosamine contamination in selected pharmaceutical products

2. Chemistry of Nitrosamine Formation

2.1. Definition of Nitrosamine

As indicated in Figure 1, nitrosamine is a class of chemicals that contain a nitroso group attached to an amine, typically represented as $R_1N(-R_2)-N=O$. Secondary, tertiary or quaternary amines may be subject to nitrosation reactions with nitrous acid, which could be produced from nitrite salts in acidic conditions. They are known to be potent genotoxic compounds in several animal species and some nitrosamines have been designated as probable or possible human carcinogens by the International Agency for Research on Cancer (IARC). Nitrosamines may be present as impurities in pharmaceutical products, and can be supplied as impurities in the active pharmaceutical ingredient (API), in methods of manufacture, from direct or indirect contamination from solvents or equipment, or as chemical degradation products during storage.

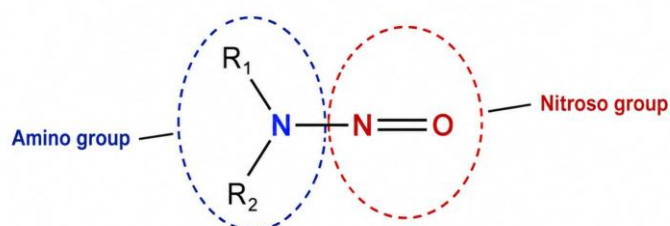


Figure 2 General Structure of Nitrosamine

2.2. Carcinogenic Potential of Nitrosamine

In the body, nitrosamines (NIs) are metabolized to form the highly reactive alkyl diazohydroxide (ADH) intermediates, primarily through the enzyme of cytochrome P450 (CYP450). These reactive species may alkylate nucleic acids, irreversibly, and thus may play a role in the mutagenic and carcinogenic activities of nitrosamines. Hepatic microsomal enzymes, notably CYP2E1 and CYP2A6, are the most important for activation of nitrosamines, and other enzymes, such as CYP3A4 and CYP1A2, may be involved[3].

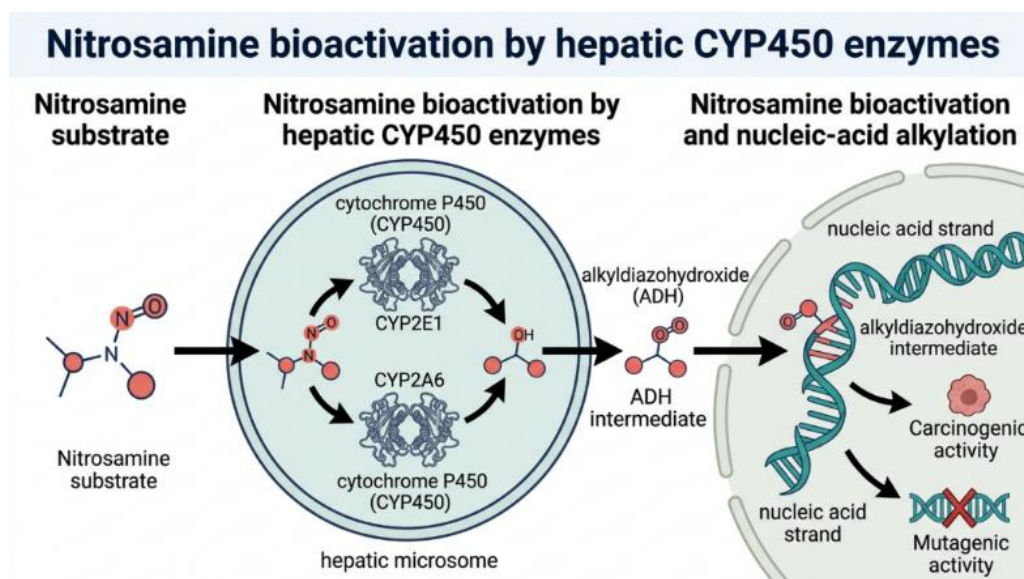
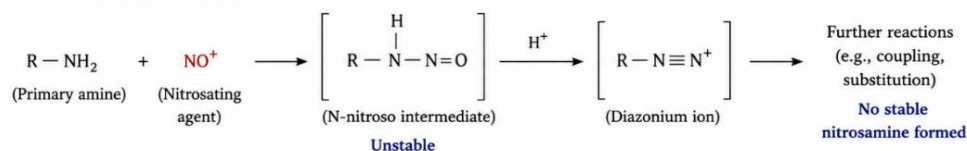


Figure 3 Metabolic bioactivation of nitrosamines by hepatic cytochrome P450 enzymes and subsequent nucleic-acid alkylation.

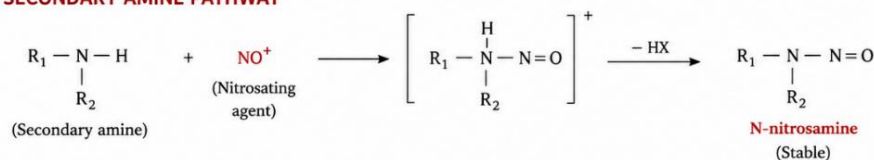
2.3. General Mechanism of Nitrosamine formation

Nitrosamine formation is primarily due to nitrosation of amine-containing compounds in the presence of appropriate nitrosating agents. Examples of common nitrosating agents are nitrous acid (HNO_2), nitrite ion (NO_2^-), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), and nitrosyl halides. Primary, secondary and tertiary amines have different reaction behavior and the pathway of nitrosation is dependent on the structure and reactivity of the amine.

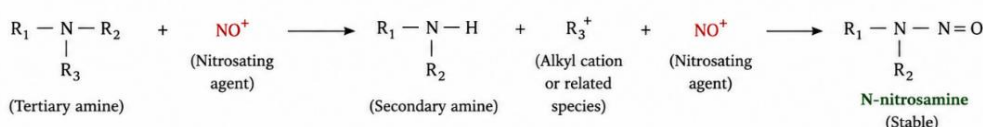
1. PRIMARY AMINE PATHWAY



2. SECONDARY AMINE PATHWAY



3. TERTIARY AMINE PATHWAY



Nitrosating agents (sources of NO^+): HNO_2 , NO_2^- (in acidic conditions), N_2O_3 , N_2O_4 , NOCl , NOBF_4 , etc.

Figure 4 Nitrosamine formation from primary, secondary and tertiary amines

Primary Amines

Primary amines (RNH_2) will easily react with nitrosating agents to produce N-nitroso intermediates. These intermediates, however, are highly unstable and tend to decompose further and form diazonium salts. However, it is noted that primary amines can undergo nitrosation reactions with the formation of the final product as an N-nitrosamine; however, they tend not to form stable N-nitrosamines.

Secondary Amines

The most important and reactive precursors for the formation of stable N-nitrosamines are secondary amines (R_2NH). They are directly N-nitrosated in the presence of appropriate nitrosating agents to form the N-nitrosamine. The reaction takes place between the amine nitrogen and an electrophilic nitrosating species, leading to the formation of the $\text{N}-\text{N}=\text{O}$ functionality that is typical of nitrosamines. The N-nitroso products formed from secondary amines are sufficiently stable to be found as nitrosamine impurities.

Tertiary Amines

Tertiary amines (R_3N) do not contain an $\text{N}-\text{H}$ bond and thus do not proceed by the same pathway as are formed from amines. Tertiary amines can undergo nitrosative dealkylation to give the corresponding

secondary amine under the proper conditions. These newly formed secondary amines can then react with nitrosating agents to form the nitrosamines. Therefore, tertiary amines can be used as a way of generating secondary amines, which can then be converted to nitrosamine impurities. Factors affecting nitrosamine formation include pH, temperature, solvent and the type of nitrosating agent. Reactive nitrosating species will usually be produced from nitrite under acidic conditions. At very low pH, though, the amine becomes proportionately more protonated, and thus unreactive. Therefore, nitrosation is expected to occur more readily when the conditions allow for the formation of the reactive nitrosating species and for the presence of the reactive amine. Overall, secondary amines are the most direct and important precursors of stable N-nitrosamines while primary amines are the primary sources of unstable N-nitroso intermediates and tertiary amines are able to form N-nitroso intermediate via nitrosative dealkylation and subsequently N-nitrosation of this intermediate [3].

2.4. Classification of Pharmaceutical Nitrosamines

In pharmaceutical industry's impurities are classified as nitrosamine impurities, which can be divided into two types:

Small-Molecule Nitrosamines: These are small molecular weight nitrosamines such as nitrosodimethylamine (NDMA), and nitrosodiethylamine (NDEA). These are not structurally related to the drug substance, and may be added to the drug product during its manufacturing process, particularly if contaminated solvents, reagents, or raw materials are used[4]

Nitrosamine Drug Substance Related Impurities (NDSRIs): NDSRIs are nitrosamines which share the same molecular structure as the active pharmaceutical ingredient (API) or have portions of the API in their structure. Their nature is therefore more or less specific to the respective API. NDSRIs may be created during the nitrosation of APIs (or degradation products with secondary, tertiary or quaternary amine functionality) in the presence of a nitrosating agent like impurities in excipients and other pharmaceutical materials.[1]

Table 1 Classification and Examples of Nitrosamine Impurities in Pharmaceuticals

Type of nitrosamine	Nitrosamine impurity	Associated API / drug
Small-molecule nitrosamine	NDMA	Valsartan
Small-molecule nitrosamine	NDMA	Metformin
NDSRI	N-Nitroso-Propranolol	Propranolol

3. VALSARTAN - ROOT CAUSE ANALYSIS

3.1. Background

Valsartan is in a drug class called angiotensin II receptor blockers (ARBs) that is used to treat chronic heart failure and high blood pressure. The use of N-nitrosodimethylamine (NDMA) was found in valsartan medicines in 2018. The contamination was associated with an API product from Zhejiang Huahai Pharmaceutical Co., Ltd. (ZHP), China. The problem was linked to a change in the

manufacturing process for the API, specifically in the ring-forming step that creates the tetrazole ring [6].

3.2. Problem Statement

The introduction of the quenchers sodium azide and sodium nitrite and the solvent DMF were thought to be responsible for the formation of NDMA in valsartan. The conditions in these processes could have led to the formation of nitrosamines. The discovery resulted in product recalls and regulatory investigations and raised concerns about the potential presence of other nitrosamines, such as NDEA, in medicines containing sartans [6].

3.3. Chemical basis of NDMA formation:

N-nitrosodimethylamine (NDMA) formation occurs through the nitrosation of dimethylamine in the presence of a nitrosating species. In the modified valsartan manufacturing process, sodium nitrite was used for quenching excess sodium azide following the tetrazole ring-forming step. Under acidic conditions, sodium nitrite generates nitrous acid (HNO_2), which acts as a nitrosating agent. Dimethylamine, identified as an impurity associated with DMF, can react with nitrous acid under these conditions to form N-nitrosodimethylamine. Therefore, the simultaneous presence of a nitrosatable secondary amine, nitrite-derived nitrous acid, and acidic conditions provides the chemical basis for NDMA formation[6]

3.4. Factors Contributing to NDMA Formation

3.4.1. Tetrazole Ring-Forming Step as the Critical Manufacturing Stage

The tetrazole ring is an important feature in the synthesis of valsartan and other sartans containing the tetrazole ring. The azide function is widely used in the synthesis of heterocycles containing N, such as tetrazole rings. In general, the tetrazole is obtained by reacting nitrile and azide compounds [6]. The evidence available suggests that the valsartan NDMA problem was related to a change in this synthetic step. ZHP has used sodium azide (NaN_3) in the manufacturing process instead of tributyltin azide as a precursor for the formation of the tetrazole ring. Sodium azide was thought to be more reactive and cost-effective inorganic alternative and yielded a greater amount of valsartan than the original patented method with the use of tributyltin azide [6].

In the 2022 review, it is also reported that contamination from nitrosamine impurities was found in valsartan produced from the modified process at the ZHP plant since July 2012. The primary motivation for modification was to increase yield and decrease waste. The modification involved the substitution of tributyltin azide by sodium azide, and the replacement of the solvent, chloroform. In the modification the use of N,N-dimethylformamide (DMF) as solvent was used instead of chloroform, and the tributyltin azide was substituted with sodium azide. Thus, the first event in the valsartan RCA has been the synthetic-route modification [8].

3.4.2. Role of Sodium Azide

To replace the source of azide, sodium azide (NaN_3) was used during the tetrazole ring-forming step. Sodium azide is found to be more reactive and economical than tributyltin azide and showed the higher yield of valsartan. Sodium azide is not very soluble in organic solvents, however, and thus can be added in excess to make up for this limited solubility in DMF [6]. Excess sodium azide is significant in the RCA since it made the need for a quenching step. In order to neutralize excess sodium azide after the tetrazole ring formation, sodium nitrite was then added to the reaction solution. Therefore, sodium azide cannot be regarded as the direct nitrosating agent that causes NDMA to form. On the contrary, the use of it in excess resulted in a process condition that required a quenching step using sodium nitrite[6].

3.4.3. Role of DMF as a Potential Amine Source

In the modified method for the preparation of valsartan, N,N-Dimethylformamide (DMF) was used as the solvent. Secondary amines are listed as possible contaminants in amide solvents. Possible secondary amine contaminants are given in amide solvents (such as DMF, N,N-dimethylacetamide and N-methylpyrrolidone). DMF can break down to dimethylamine under certain reaction conditions, then the dimethylamine can react with nitrous acid to create NDMA [8].

3.4.4. Role of Sodium Nitrite and Quenching Operation

The quenching reaction is the most important initial chemical reaction in the pathway of valsartan NDMA formation. After the step of the formation of tetrazole ring, excess sodium azide was quenched with sodium nitrite (NaNO_2). Sodium nitrite can transform into nitrous acid (HNO_2) an aggressive nitrosating agent under acidic conditions. That sodium nitrite used for quenching excess sodium azide can create nitrous acid which react with dimethylamine linked to DMF can go through a process called nitrosation to make NDMA [8].

3.4.5. Role of Acidic Reaction Conditions

The formation of nitrous acid from sodium nitrite is favored under acidic conditions. Reaction conditions are therefore an important contributing factor in the valsartan RCA. Nitrosation is most pronounced under acidic conditions, with an optimal pH of approximately 3–4 [8].

3.5. Five-Why Analysis of Valsartan NDMA Formation

To systematically identify the underlying factors contributing to NDMA formation during valsartan manufacturing, a Five-Why analysis was used to trace the root-cause pathway step by step.

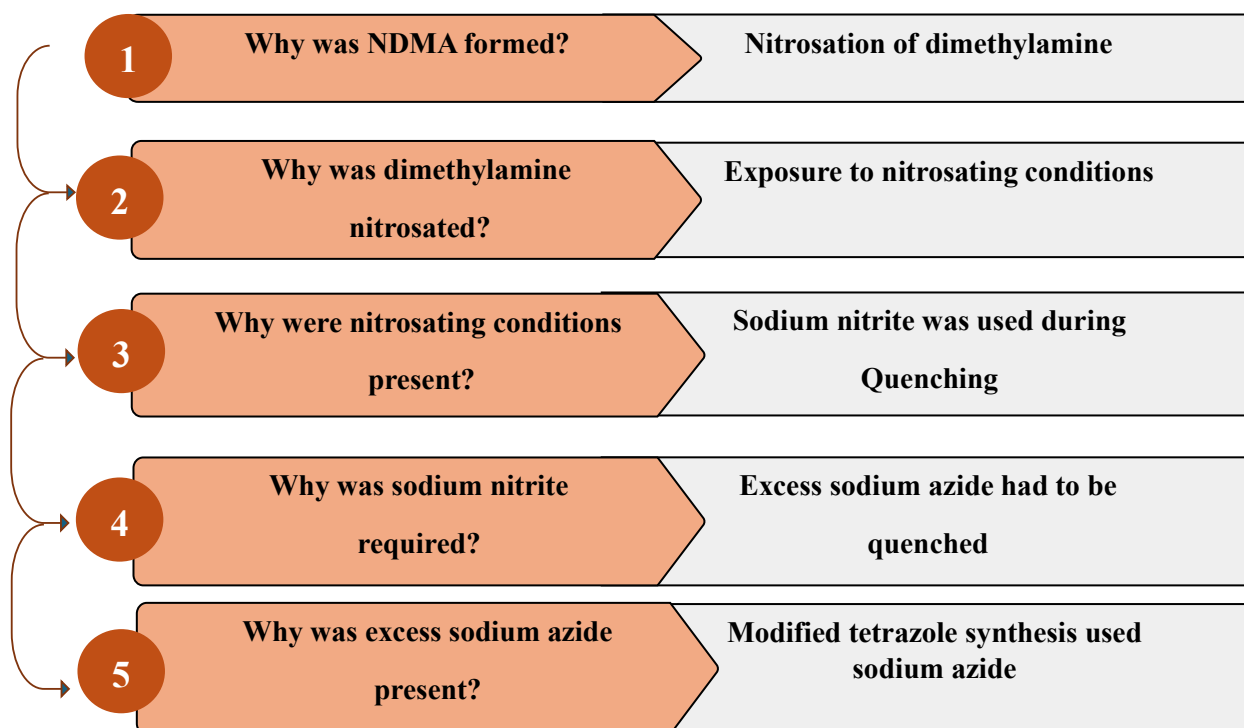


Figure 5 Five-Why analysis for NDMA formation in valsartan API

3.6. Summary of Root/Contributing Causes

- ❖ RC1. Modification of the tetrazole ring-forming step, replacing tributyltin azide with sodium azide
- ❖ RC2. Excess sodium azide requiring a quenching step due to limited solubility in DMF

- ❖ RC3. Sodium nitrite quenching under acidic conditions, generating nitrous acid as the nitrosating species
- ❖ RC4. Presence of dimethylamine linked to the DMF solvent, which acted as a nitrosatable amine

Overall Root Cause Determination: NDMA was formed from valsartan via reaction pathway RC1, which was required to form RC2, which provided the nitrosating conditions of RC3, which then reacted with the amine source from RC4.

4. METFORMIN – ROOT CAUSE ANALYSIS

4.1. Background

Metformin is a commonly used oral antidiabetic agent used in the treatment of type 2 diabetes mellitus. In 2019, N-nitrosodimethylamine (NDMA) was found in metformin drug products and concerns have been raised about its presence and potential formation during drug manufacture. In the study by Fukuda et al. (2023), 1.9% of 881 batches of metformin contained NDMA concentrations exceeding the provisional limit of 43 ppb. The excessive occurrence of NDMA was especially seen in the Osaka manufacturing site, which led to the investigation of the factors leading to its formation. This investigation then turned to the API metformin, its manufacturing process, and environmental factors which could contribute to the formation of NDMA [9].

4.2. Problem Statement

The presence of NDMA in metformin drug products which exceeds the provisional acceptable limit caused concern because of its potential to be formed during the manufacturing process. The excessive NDMA found in products produced at the factory in Osaka, but not in products produced in other factories, indicated that a systematic investigation into the root cause was needed. The purpose of this investigation was to establish where NDMA is from and what materials, environmental and process factors contribute to the formation of NDMA [9].

4.3. The chemical basis of the formation of NDMA

N-nitrosation of the residual dimethylamine (DMA) in the metformin API with nitrosating species formed by reaction of N_2O_4 in the presence of atmospheric NO_2 is the chemical basis of the NDMA in drug products. From the fluidized-bed granulation: API is in contact with a lot of heated air in which NO_2 is present in the atmospheric air, which is ideal for the nitrosation reaction to happen. Fukuda et al. proved that the higher the content of DMA, the concentration of NO_2 , the longer the reaction time, the higher the temperature, the greater the amount of NDMA formed. These authors suggested that the nitrosation process might involve the formation of nitrosonium ions, NO_2 , but admitted that radical and surface reactions might also play a part and that the detailed mechanism may still be fully established [9].

4.4. Factors Contributing to NDMA Formation

4.4.1. Content of dimethylamine (DMA) in the API

It was determined that an important precursor for the formation of NDMA in the metformin active pharmaceutical ingredient (API) was dimethylamine (DMA) that was present in the API as a trace impurity. The investigation revealed that the majority of the products made with API from supplier A contained significantly more DMA (1008.9 ppm) than the API from supplier B (51.2 ppm), and that the average DMA concentration in the products was higher when API from supplier A was used. Similar results were observed in model experiments, which generated 93.8 ppb NDMA with API containing 1768.3 ppm DMA and 4.3 ppb NDMA with API containing 56.5 ppm DMA under the same conditions

(50 ppb NO₂, 65 °C and 150 min). The study also revealed that only some 0.003–0.005% of the DMA was required to react to yield up to the provisional standard of NDMA. Therefore, concentration of DMA in API is important material-related factor, and the presence of DMA in API when exposed to atmospheric NO₂ during fluidized-bed granulation can trigger the formation of NDMA. The amount of NDMA formed was considerably affected by the concentration of DMA, NO₂, reaction time and temperature[9].

4.4.2. Atmospheric NO₂ concentration

Nitrogen dioxide (NO₂) in the atmosphere was determined as one of the most significant factors related to the environment or to the process that leads to the formation of NDMA in metformin drug products. During the investigation, it was noticed that the difference existed between two manufacturing sites. The Osaka factory, which was responsible for the production of batches that were above the provisional NDMA standard, was correlated with higher NO₂ concentrations in the atmosphere compared to the Yamagata factory. Metformin batches were analyzed for NDMA that had a positive trend with the amount of NO₂ in the atmosphere. The Osaka site had a higher monthly average concentration of atmospheric NO₂ than the Yamagata site, and the concentration of NO₂ in the air was not higher than the provisional standard even in the months with the lower concentration of NO₂ in the air. The correlation between monthly average NO₂ and NDMA was however moderate ($r = 0.59$), as these NO₂ measurements were monthly average values 1.2–2.5 km away from the actual granulator and not measurements of the air inside the granulator.

They took more direct measurements to see how much NO₂ was present when the air was fed into the fluidized-bed granulator at the Osaka factory. NO₂ levels found in the incoming air were between 5-60ppb, with a peak NO₂ concentration of 72.1 ppb. The API was directly exposed to this air during fluidized-bed granulation, which was the reason for these measurements. In order to confirm the role of NO₂, model experiments were conducted in which metformin API was treated with 0, 25, 50 and 100 ppb NO₂ for 150 minutes at temperature of 65 °C. As the concentration of NO₂ increased, so did the amount of NDMA formed, and importantly, the amount of NDMA formed was in the range of 5-60 ppb corresponding to the NO₂ concentration measured in the actual granulator. This illustrated that low concentrations of atmospheric NO₂ can be sufficient to allow the conversion of DMA present in the API into NDMA. The study concluded that atmospheric NO₂ entering the fluidized-bed granulator was a significant factor in the NDMA formation and that the effect of NO₂ occurred in conjunction with API content of DMA and the heated, prolonged exposure conditions during granulation. Therefore, decreasing the concentration of the NO₂ in the air going into the granulator was considered to be an effective way of minimizing the amount of NDMA formed [9].

4.4.3. Fluid Bed Granulation

A fluidized-bed granulation was chosen as the key manufacturing process where conditions for the formation of NDMA could exist. Here, a vast amount of air is allowed to hit the metformin API while the temperature is raised. The investigators therefore concentrated on this step, since it is the one where the DMA present in the API comes in direct contact with the NO₂ brought into the granulator with the incoming air. The study hypothesized that trace levels of API of the drug metformin would be nitrosated in the presence of atmospheric NO₂ during fluidized-bed granulation and thus form NDMA.

The wet granulation process used for the manufacturing of metformin consisted of granulating the API (wet granulation) in a fluidized-bed granulator, followed by drying and tablet compression. The granulation process was carried out by feeding the conditioned air at the quality of 65 °C and 50%RH, at the rate of around 60 m³/min for 150 minutes. As a result, this process exposed the API to a significant amount of hot air with a high amount of atmospheric NO₂ for an extended period of time.

This process was confirmed by the model experiments that simulate the granulation conditions. In the presence of NO₂ under conditions similar to those used in fluidized bed granulation, NDMA formation was detected when metformin API was exposed. Moreover, reaction time and the concentration of NO₂ both increased the amount of NDMA and heating accelerated the conversion of DMA to NDMA. The formation of NO₂ was confirmed and the NO₂ concentration measured was close to that observed in the granulator, which was enough to promote NO₂ formation.

Thus, the process environment of fluidized-bed granulation made it possible to combine favourable temperature and exposure time with the two important chemical factors, DMA in the API and atmospheric NO₂. The study therefore suggested reducing exposure to NO₂ in the atmosphere, granulation time, and granulation temperature as possible ways to decrease the amount of NDMA formed [9].

4.4.4. Granulation Temperature

Granulation temperature (GT) was selected as a process-related parameter that affects the formation of NDMA in granulation of metformin drug product in a fluidized-bed granulator. In this study, temperature was chosen as the primary parameter since during granulation, the metformin API along with the DMA (in trace amounts) is exposed to heated air containing atmospheric NO₂. In such cases, the heat may speed up the conversion of DMA to NDMA. The impact of temperature was, in particular, investigated through a model experiment, where metformin API was treated with 50 ppb NO₂ for 150 minutes at two temperatures 22 °C and 65 °C. The NDMA was formed at room temperature (22 °C); more NDMA was formed at the higher temperature of 65 °C. This showed that the rate of DMA to NDMA was accelerated when heated in the presence of NO₂. The effect of temperature is thus linked with the reaction kinetics of the nitrosation process. The reaction between the DMA precursor and the nitrosating species can also take place more easily, when the API is exposed to nitrosating species in the air, while it is being heated during granulation process. It may therefore be expected that the increase in granulation temperature would lead to an increase in the amount of NDMA produced. The study showed that the concentration of NO₂, reaction time and reaction temperature have significant impacts on the quantity of NDMA generated, in addition to the DMA concentration in the API. Therefore, granulation temperature is an important factor that contributes to the formation of NDMA, but it is not a source. The importance is that the DMA and NO₂ from the atmosphere are present under heated situations to create favorable conditions for N-nitrosation. Therefore, one of the measures that may reduce the formation of NDMA is to reduce or control the granulation temperature, if possible without compromising product quality [9].

4.4.5. Granulation Time

For fluidized bed granulation, granulation time was determined as a critical process-related variable for the formation of NDMA. Fukuda et al. studied reaction time in a model system of metformin API and atmospheric NO₂. Under the following conditions, the amount of NDMA generated steadily increased from 30 to 90 and 150 minutes of reaction time at a constant NO₂ concentration of 50 ppb and temperature of 65°C. The observed increase over time suggests that extended exposure of NO₂ during the granulation step of exposure to DMA in the metformin API affords more opportunity for the formation of NDMA. The study also found that reaction time, in addition to NO₂ concentration and temperature, are factors determining NDMA formation. Controlled or reduced granulation time, when possible, can help reduce the amount of NDMA formed in the actual fluidized-bed granulation process because the total granulation process exposure time was around 150 minutes of conditioned air. Therefore, too long a granulation time is a contributing process factor due to longer periods of contact between DMA and atmospheric NO₂ that are favorable for the formation of NDMA [9].

4.5. Fishbone (Ishikawa) Analysis of NDMA Formation in Metformin

Fishbone (Ishikawa) cause-and-effect analysis was used to systematically discover and categorize the factors that can affect the formation of NDMA in drug products containing metformin. The factors that may have contributed have been broken down into the areas of Materials, Methods, Equipment, Environment, People and Measurement/monitoring. Based on the study results by Fukuda et al., (2023), the presence of dimethylamine (DMA) in the API of metformin, atmospheric NO₂ and fluidized-bed granulation conditions (granulation time and temperature) were of special interest. The linkage that has been discovered between the cause and the effect is shown on the fishbone diagram below.

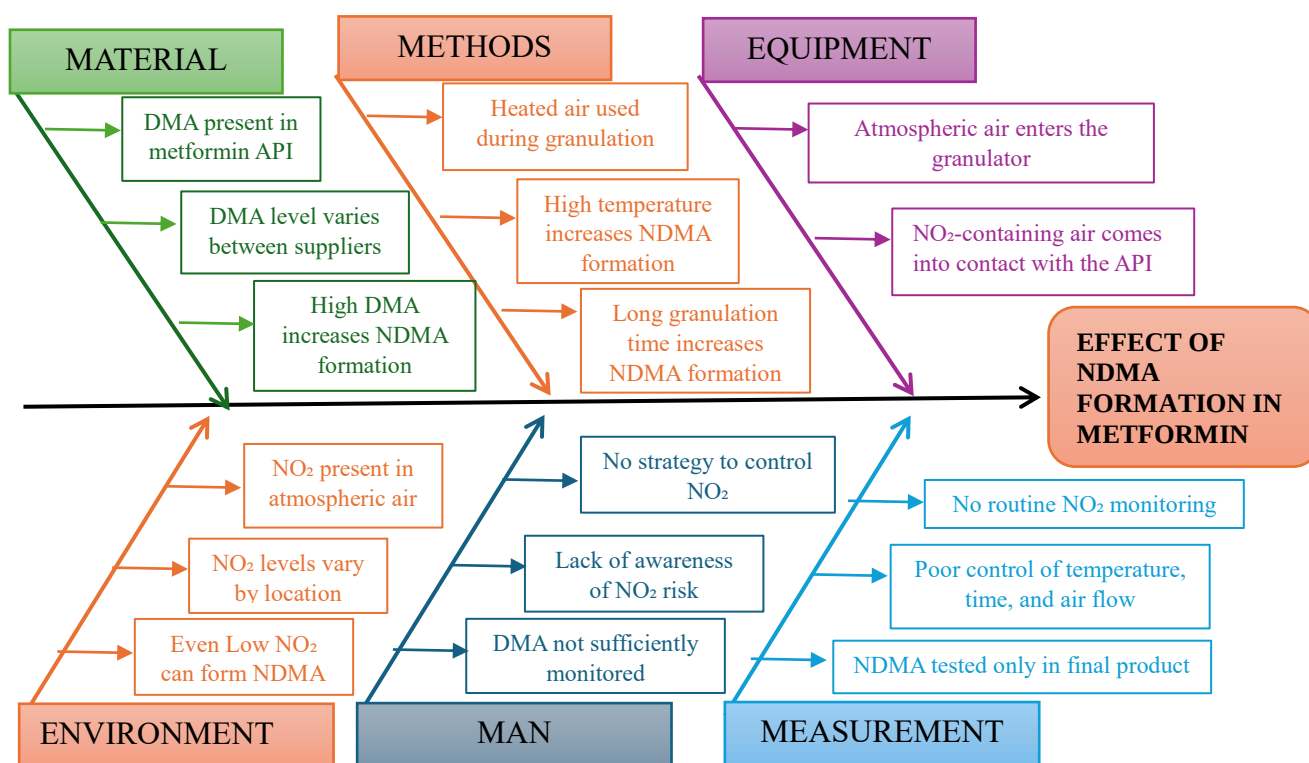


Figure 6 Fishbone (Ishikawa) analysis of NDMA formation in metformin.

4.6. Summary of Root/Contributing Causes

- ❖ RC1. Dimethylamine present as a trace impurity in the metformin API, with concentration varying by supplier
- ❖ RC2. Atmospheric NO₂ entering the fluidized-bed granulator during processing
- ❖ RC3. Granulation conditions (temperature ~65 °C, time ~150 min) favoring the nitrosation reaction

Overall Root Cause Determination: NDMA formation in metformin resulted from the interaction of RC1 and RC2 under the granulation conditions of RC3.

5. RIFAMPICIN – ROOT CAUSE ANALYSIS

5.1. Background

Rifampicin is an important antibiotic used in the treatment and prevention of tuberculosis. Pharmaceutical products containing rifampicin have been reported to contain an impurity of 1-methyl-

4-nitrosopiperazine (MNP), a genotoxic nitrosamine impurity. In the study by Witkowska et al. the presence and formation of MNP in rifampicin products including multi-component formulations was investigated. The authors reported that MNP contamination in the rifampicin is generated during its manufacture and that through forced degradation experiments two pathways for the generation of MNP were identified: degradation of rifampicin and oxidation of 1-amino-4-methylpiperazine, which is a product of the rifampicin synthesis route. The study also proved that forming MNP is not a phenomenon limited to the manufacturing process but can also occur during storage or under stress. The MNP content in the four component rifampicin product was found to be increasing during its storage at 25 °C/60%RH and 40 °C/75%RH in accelerated stability studies [10].

5.2. Problem Statement

The presence of MNP in products containing rifampicin led to concerns about the potential for the formation of MNP during the manufacture, storage and degradation of rifampicin. The study demonstrated that MNP can be formed under elevated temperatures resulting in high temperature in some analytical procedures that can also induce MNP formation. The authors thus examined the formation of MNP under various stress conditions, namely thermal, oxidative, alkaline and acidic, to find the factors responsible for the formation of MNP in products containing rifampicin. The investigation showed that conditions of oxidative nature and thermal stress in the presence of atmospheric oxygen most closely correlate with the formation of MNP. The study also revealed that the storage temperature, storage time and storage humidity were positively associated with the amount of MNP [10].

5.3. The Chemical Basis of MNP Formation

1-amino-4-methylpiperazine is thought to be responsible for the chemical basis of MNP formation in rifampicin, which is linked to the degradation of the rifampicin molecule. The researchers found that 1-amino-4-methylpiperazine can be a residual product of the drug's synthesis and is present in the drug as part of the synthesis process for rifampicin. In appropriate conditions, this amine can be oxidized to MNP. The study suggested another route which is the degradation of rifampicin itself. The structure of the rifampicin can be broken down in the presence of acid to a hydrazine derivative (1-amino-4-methylpiperazine) of a hydrazone type. This intermediate can then be oxidized to form MNP. The scheme of proposed degradation comprises hydrazone hydrolysis and oxidation. The authors further suggested an alternate route for MNP formation at higher temperatures. In this pathway, hydrolysis of the rifampicin could take place without the hydrazone structure being completely broken down, and oxygen is needed in the oxidative pathway. Oxygen can form a bond with the rifampicin structure prior to or after breaking, which can finally be a contributor for the formation of MNP [10].

5.4. Factors Contributing to MNP Formation

5.4.1. Presence of 1-Amino-4-Methylpiperazine in Rifampicin

An important factor identified in the formation of MNP was the presence of 1-amino-4-methylpiperazine. The compound was not detected in any of the 1-methylpiperazine APIs or products, while the rifampicin API was detected in the four-component rifampicin product. This observation confirmed 1-amino-4-methylpiperazine role as a precursor involved in MNP formation. The study also showed that the amount of 1-amino-4-methylpiperazine would be elevated in acidic conditions due to the break down of rifampicin to produce 1-amino-4-methylpiperazine. It was subsequently oxidized to produce MNP. [10].

5.4.2. Acid-Induced Degradation of Rifampicin

Acidic conditions played a role in the formation of MNP by favouring the degradation of rifampicin. Under acidic conditions, the primary degradation products found were 3-formyl rifampicin SV and 1-

amino-4-methylpiperazine. The degradation product 1-amino-4-methylpiperazine was observed to increase in acidic pH, supporting the degradation of rifampicin can lead to the generation of the amine precursor necessary for subsequent formation of MNP. The authors suggested the hydrolysis of the hydrazone structure of Rifampicin in an acidic medium would give a hydrazine derivative which is represented as 1-amino-4-methylpiperazine. This can then be oxidized to MNP [10].

5.4.3. Oxidative Conditions

It was found that oxidation is an important process in the conversion of the amine precursor into MNP. Forced degradation experiments showed a considerable increase of the concentration of MNPs and oxidizing conditions. For the four component rifampicin drug product, treatment with 3% hydrogen peroxide resulted in a large increase in MNP (2920 ppm) compared to the control sample (7.07 ppm). The study also revealed that the presence of an oxidizing agent led to an increase in the formation of MNP from 1-amino-4-methylpiperazine. [10].

5.4.4. Temperature

Another major parameter that played a role in the formation of MNP was temperature. Thermal stress experiments showed that the MNP of products containing rifampicin increased when the products were under the influence of high temperature in air. It was reported that increase in storage temperature resulted in an increase in MNP formation in the study. Their experiments showed that thermal stress in an airy atmosphere resulted in a large increase in MNP, which suggests that temperature alone is not the sole determinant of the production of MNP, and that oxygen present in the air is also involved in the process [10].

5.4.5. Oxygen and Humidity

O₂ and humidity in the atmosphere were found to be significant environmental parameters for the formation of MNP by temperature. The study showed that the temperature-induced degradation of rifampicin to MNP could be inhibited when access to oxygen and humidity from the air was prevented. The authors reached this conclusion after observing these facts; they suggested that oxygen, humidity, and acidic conditions are responsible for rifampicin degradation to MNP. Accelerated stability studies also revealed that the content of MNPs increased with the increase of temperature, humidity, and storage time. [10].

5.4.6. Rifampicin Degradation

Another pathway for the formation of MNP is the degradation of rifampicin. Thermal, oxidative, alkaline and acid hydrolysis conditions were used in the forced degradation experiments. A considerable rise in MNP was seen especially when exposed to oxidative stress and thermal stress in an airy atmosphere. The study suggested that the degradation of rifampicin could be responsible for the formation of 1-amino-4-methylpiperazine followed by the oxidation of 1-amino-4-methylpiperazine to form MNP. [10].

5.5. Fault Tree Analysis of MNP formation in rifampicin

As a result, Fault Tree Analysis (FTA) was chosen for evaluation of the root causes of MNP formation in rifampicin as there were more than one possible pathway identified by the reference. The study also showed that MNP can be formed during the oxidation of the synthesis-derived 1-amino-4-methylpiperazine and suggested that MNP is a degradation product of rifampicin, which might be the result of the acidity, temperature, oxygen, humidity and time spent in storage. Therefore, FTA was deemed applicable to the task of systematically portraying the relationship between the top event, intermediate pathways and contributing factors[10].

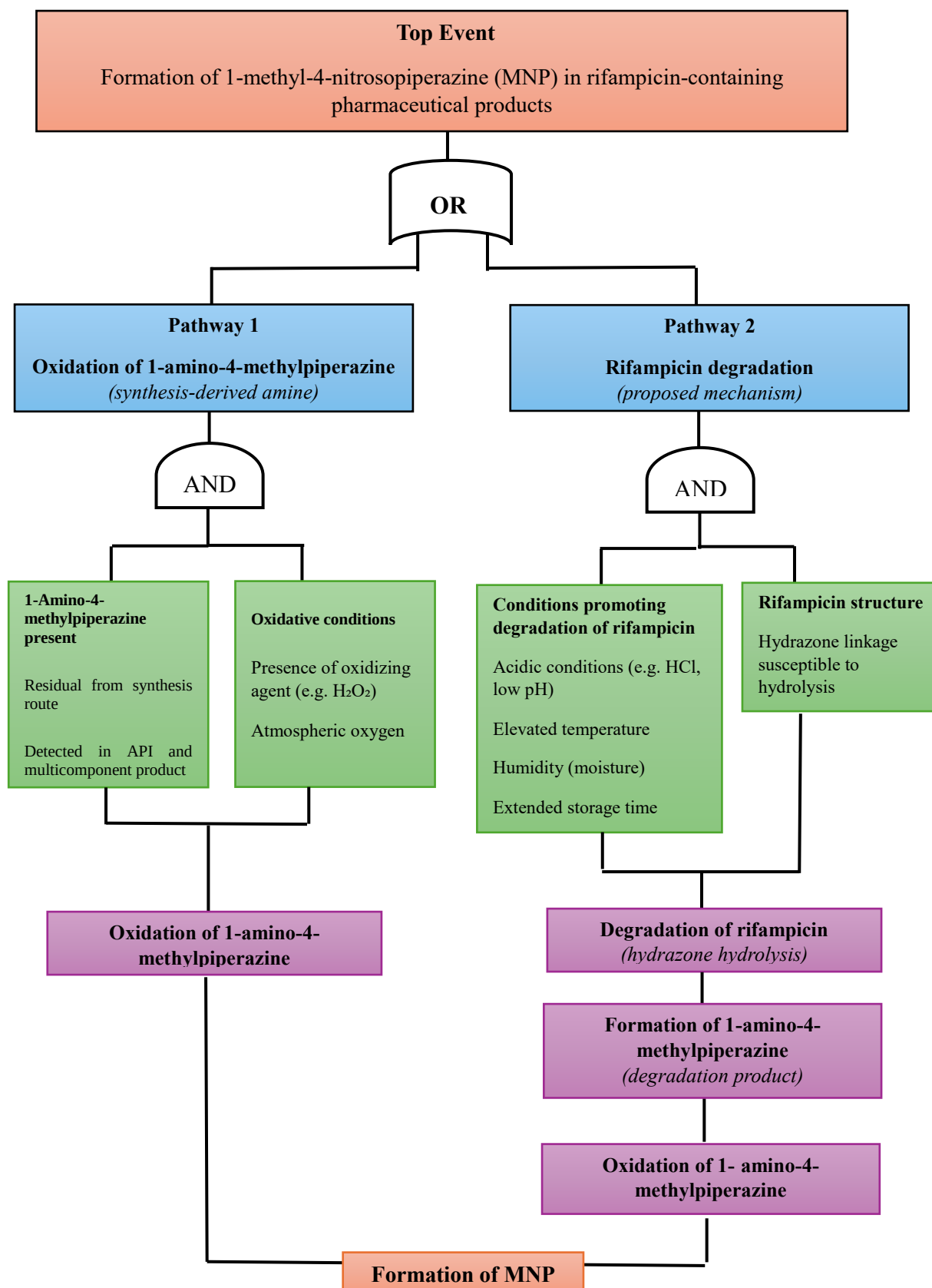


Figure 7 Fault Tree Analysis (FTA) of MNP formation in rifampicin-containing products. OR indicates alternative pathways, while AND indicates combined conditions required for the pathway.

5.6. Summary of Root/Contributing Causes

- ❖ RC1. Presence of 1-amino-4-methylpiperazine in API/products synthesized from it
- ❖ RC2. Acidic or thermal degradation of rifampicin forming 1-amino-4-methylpiperazine (proposed mechanism)
- ❖ RC3. Oxidative conditions promoting conversion of 1-amino-4-methylpiperazine to MNP
- ❖ RC4. Storage/environmental conditions (temperature, humidity, duration) increasing MNP formation

Overall Root Cause Determination: MNP formation in rifampicin resulted from either RC1 or RC2 generating the amine precursor, with RC3 driving its conversion to MNP, compounded by RC4 during storage.

Table 2 Comparative framework for Nitrosamine Root causes

DIMENSION	VALSARTAN	METFORMIN	RIFAMPICIN
Nitrosamine formed	NDMA	NDMA	MNP
Primary Source	API manufacturing process	Manufacturing process/ environment	API precursor or Degradation
Nitrosating Agent source	Sodium nitrite (azide quenching step)	Atmospheric NO ₂ (Granulation air)	Atmospheric/oxidative O ₂ acting on amine precursor
Amine precursor	Dimethylamine (from DMF)	Dimethylamine (trace API impurity)	1-amino-4-methylpiperazine(synthesis residual or degradation pathway)
Key contributing factor	Azide route modification, DMF solvent, acidic quench conditions	DMA content variability by supplier, NO ₂ ingress, Granulation temperature, time	Acidic/thermal degradation, oxidative exposure, storage, temperature, humidity
RCA tool applied	Five- Why analysis	Fishbone(Ishikawa) Analysis	Fault tree analysis(FTA)
Rationale for tool choice	Single linear causal chain	Multi categorical contributing factor	Two converging, non-linear pathways

6. EVIDENCE BASED MITIGATION STRATEGIES

Unacceptable levels of nitrosamine impurities in active pharmaceutical ingredients (APIs) and drug products are recommended to be identified and prevented by drug manufacturers according to the U.S. Food and Drug Administration (FDA). Final guidance was issued in September 2024, which lists some potential root causes, risk assessments, confirmatory testing and use of controls/other measures for impurities related to nitrosamines. The different stages of FDA's regulatory process can be categorized into three main categories: assessing potential risk, testing confirmation, and implementing controls and/or regulatory changes to prevent or reduce potential risk. reduce the impurity [11].

6.1. Valsartan – Evidence-Based Mitigation Strategies

The EMA's Article 31 evaluation of sartans led to the determination that exposure to NDMA and NDEA was a quality concern, and examined the potential sources and solutions for valsartan and other sartans containing tetrazoles. Evaluation of the formation of nitrosamines has been correlated with the different combinations of amine sources, nitrite, reaction conditions, and manufacturing methods. [12,13]

6.1.1 Control of Sodium Nitrite and Azide-Quenching Conditions

One of the recommendations that the key EMA made was to not use sodium nitrite (NaNO_2) until the API has been separated from the aqueous phase in the work-up process. The sodium azide can be quenched by sodium nitrite, but if amino species are appropriate, and conditions are favourable, nitrosamines can be formed. Separating the API containing phase prior to the nitrite quenching can therefore help to minimize the potential for the forming of nitrosamines. If sodium azide is employed in the synthesis, the process shall be modified and the process shall be qualified or the presence of azide shall be sufficiently eliminated by using the appropriate sensitive and validated analytical method. EMA also suggests batch data that shows either the absence of azide or good control of it in the API. If nitrite is employed outside of the API, solvents recovered from the API should not be recovered for reuse unless allowing for the possibility of nitrosamine contamination is made a part of the adequate precautions. [12]

6.1.2 Alternative Reaction Conditions and Materials

Based on the EMA assessment, alternative reaction conditions is identified as a potential mitigation strategy. This can be done with amide solvents (DMF, DMA or NMP as appropriate) as well as secondary or tertiary amines or less reactive amine bases. It is designed to eliminate or reduce the levels of reagents and conditions that might lead to the formation of nitrosamines in the product. Hence, it is recommended to consider the chemical compatibility of starting materials, solvents, reagent and reaction conditions during the manufacturing-process review. The EMA does not suggest that the public consider the drugs as NMPs, but rather recommends that companies look at the manufacturing methods that may be used to create the drug, and ensure that no NMPs are present in the drug, while FDA will expect companies to know and control the sources of NMPs that may develop during the drug's manufacturing process. [13,14]

6.1.3 Change in Reaction Sequence and Purification

The other possible mitigation measures identified by EMA were modifications of the sequence of the reactions. Introducing the tetrazole The subsequent synthetic steps may have the opportunity to remove nitrosamine impurities if the precursor chain (moiety) is composed of many steps. Further crystallization may also be considered in addition to the purification in order to achieve an adequate level of impurity removal, if it can be demonstrated analytically that this will be accomplished. Alternative quenching agents for the removal of azide were also suggested by EMA as a possible replacement for sodium nitrite in the azide destruction process. This is not an automatic acceptance, but

should be demonstrated by process understanding and analytical data to show the intended process impurity is under acceptable control as is the nitrosamine risk. [12]

6.1.4 Analytical Testing and Lifecycle Control

An integral part of valsartan nitrosamine mitigation is the analytical testing. While FDA's current guidance suggests confirmatory testing if there is a nitrosamine risk, EMA recommends using appropriate analytical techniques and supporting batch data to show adequate control. Thus, the documented mitigation pathway for valsartan can be summarized as follows: identification of conditions that create the nitrosamine pathway; optimization of sodium nitrite/azide quench operations; control of solvents and amine containing reagents; optimization of reactions sequence/purification; validated analytical testing; and continued control during the manufacturing lifecycle. [1,12,13]

6.2. Metformin – Evidence-Based Mitigation Strategies

DMA was found in some extended-release metformin products by FDA above its acceptable intake limit, and FDA has asked extended-release metformin manufacturers to assess the risk of high levels of NDMA and to test products that are suspected to contain excessive NDMA before each new batch was marketed in the U.S. Further, FDA reported that none of the immediate release products it tested contained any amount of NDMA, and they might have been “manufactured without the impurity.” [15]

6.2.1 Product and Process Risk Assessment

Structured assessment of the potential source of NDMA is the first mitigation step done for metformin. The full manufacturing system and product formulation should be considered when making an investigation of the impurity, FDA said, but it was not FDA's position that the metformin API was the primary source based on the information available during FDA's investigation[15]. Whereas, FDA general guidance on nitrosamines states that one may need to evaluate potential sources of the drug substance, drug product, manufacturing process and other relevant materials/conditions. So, metformin mitigation should be linked to the risk that has been identified, and not to the source of the nitrosamines in all metformin products. [1]

6.2.2 Confirmatory Testing and Batch-Release Control

For the extended release metformin products, the manufacturer was specifically required by the FDA to test the product if it was considered to be a risk prior to each batch being sold in the U.S. market. The manufacturers had to report NDMA found above the acceptable limit of intake, and they were not to ship the contaminated batch to FDA. This provides direct regulatory support for confirmatory testing and batch-release control as mitigation for metformin use. Furthermore, FDA has published a test to detect the presence of NDMA in metformin containing products. Otherwise, manufacturers are free to adopt their own method or the FDA method, but if they are going to use the results for a quality assessment or for a regulatory submission, then the method needs to be validated by the manufacturer. [15,16]

6.2.3 Control of Materials and Manufacturing Factors

The following FDA mitigation framework recommends assessment of material and manufacturing issues that may result in nitrosamine formation: This will include the discussion of nitrite impurities in excipients and material/supplier controls when identified as a source during the risk assessment. If a causal factor is identified, then, evidence-based mitigation measures for metformin could include API and excipient source evaluation, manufacturing and storage conditions assessment (if applicable), supplier/material control and confirmatory testing for at-risk excipients or batches and/or change in the excipient and/or API (if applicable). The measures should be chosen based on the source of NDMA recorded. [1,11,15]

6.3. Rifampicin – Evidence-Based Mitigation Strategies

The nitrosamine impurity 1-methyl-4-nitrosopiperazine (MNP) has been found in rifampicin (also known as rifampicin). FDA investigated MNP in rifampicin products and set the acceptable-intake considerations, while the manufacturers and the regulators investigated the source and took measures/potential measures to reduce or eliminate the impurity [17,18]. EMA also says they looked into 1-nitroso-4-methylpiperazine in medicines containing rifampicin in Europe. As a precautionary measure, national competent authorities have asked the manufacturers of medicines which contain rifampicin to test their authorised medicines before dispensing until the investigations are completed and corrective measures are taken. [18]

6.3.1 Specific Analytical Testing for MNP

An LC-ESI-HRMS method was developed by FDA for the determination of MNP in the drug substance of rifampicin and drug products. The Method has been developed and validated for sensitive detection/quantitation with reported limits of detection and quantitation suitable for the intended analytical application. This is an aspect that should be addressed directly through regulatory support for specific analytical monitoring for MNP as part of the mitigation strategy. [19]

6.3.2 Batch Testing and Control Against Applicable Limits

FDA has published laboratory testing results for rifampicin and has determined an acceptable intake level of MNP. The acceptable-intake information that is currently available to FDA is 400 ng/day for MNP with rifampicin and the interim-control information is 5 ppm (interim control limit) for rifampicin injection and capsules during the period of implementation [5,18]. In addition, FDA's action with regard to rifampicin further underscores the importance of conducting batch-specific testing once an identification of a nitrosamine risk has been made. Manufacturers were asked to provide test data; FDA used test data in guiding regulatory action when conducting investigations. [17,18]

6.3.3 Reduction or Elimination of MNP

The manufacturers and the FDA were investigating the source of the MNP in the drug and attempting to reduce or eliminate the impurity, therefore the cause of this impurity in the drug has not yet been determined. Thus, investigation of any factors leading to the formation of the MNP and implementing of any scientifically valid change in the impurity should be part of the mitigation process if MNP is detected [17]. The analytical method document provided by FDA also reports that a starting material used in the synthesis of rifampicin is 1-amino-4-methylpiperazine that can form MNP under certain circumstances. This allows for an acknowledgement that the source of MNP is process conditions and starting material chemistry in evaluating the source of MNP. [20]

7. COMPARATIVE MITIGATION STRATEGIES

The root causes for each drug product are different and need to be addressed in each case by drug-specific mitigation strategies, depending on the nature and source of the nitrosamine impurity. While the overall mitigation steps include risk assessment, management of contributing factors, analytical testing and ongoing monitoring, the individual steps vary depending on the causal pathway. The modifications in manufacture, control of sodium nitrite and sodium azide, control of solvents/reagents and purification are emphasized for valsartan. Mitigation for metformin includes control of contributing material and manufacturing factors along with confirmatory and batch-release testing. Investigation and control of contributing manufacturing or material factors, along with analytical testing and on-going monitoring, are important in the case of rifampicin. Table 3 summarizes the acceptable intake of the three drugs, mitigation strategies, and analytical/control measures.

Table 3 Evidence Based Mitigation Strategies

Drug	Nitrosamine	Acceptable Intake (AI)	Mitigation Strategy	Analytical / Control Measures
Valsartan	NDMA	96 ng/day	Modification of manufacturing conditions; control of sodium nitrite and sodium azide; solvent/reagent control; modification of reaction sequence; purification/purge	Validated nitrosamine testing; process and batch monitoring
Metformin	NDMA	96 ng/day	Control of contributing materials/excipients; manufacturing-process modification where justified; appropriate product/process controls	Confirmatory testing of at-risk batches; batch-release testing; validated analytical method
Rifampicin	MNP	400 ng/day	Investigation and modification of contributing manufacturing/material factors; measures to reduce or eliminate MNP	MNP-specific analytical testing; batch/release control; continued monitoring

8. Conclusion

Nitrosamine impurities are a concern for pharmaceutical quality and patient safety because of their ability to damage DNA and cause cancer. This review shows that nitrosamine formation can happen in ways depending on the drug how it is made and how it is stored. By comparing valsartan, metformin and rifampicin the review reveals pathways and factors that lead to impurity formation in each case. To understand what causes these impurities root cause analysis was used with methods suited to the type of problem. For valsartan the Five-Why analysis helped uncover the step-by-step reasons behind NDMA formation. In the case of metformin, the Fishbone (Ishikawa) diagram was used to organize and analyze the material, environmental and process-related factors connected to NDMA. For rifampicin Fault Tree Analysis was applied to map out all routes that led to MNP formation. These findings show that controlling nitrosamine precursors is essential. It also highlights the need to carefully manage manufacturing conditions ensure raw materials are safe and keep factors under control. Validated testing methods and ongoing monitoring play a role in detecting impurities early. Combining knowledge with structured root cause analysis and science-based solutions creates a strong framework for identifying and managing nitrosamine risks. The drug-specific strategies outlined in this review can help improve how we prevent nitrosamine impurities. This approach supports quality in pharmaceutical products and helps protect patients by reducing exposure, to harmful contaminants.

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