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NAVIGATING NITROSAMINE IMPURITIES IN PHARMACEUTICAL INDUSTRY

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Abstract:

The discovery of N-nitrosamine impurities in medications and the subsequent appearance of nitrosamine drug substance related impurities (NDSRIs) have presented both regulators and drug product producers with significant challenges. Global regulators have lately taken action in response to the unexpected discovery of nitrosamine impurities in human medicines in order to comprehend the hazards these contaminations pose to patients and to limit their presence. NDSRIs are primarily connected to drug product reactions, which adds a special complication. There are known to be over 300 nitrosamines, several of which are powerful mutagenic carcinogens. The discovery of N-nitroso-dimethylamine (NDMA) in valsartan from one manufacturer in 2018 alerted regulators to the existence of nitrosamines in EU pharmaceuticals for the first time. N-nitroso dimethylamine (NDMA) and N-nitroso-diethylamine (NDEA) garnered significant attention. Since then, these have been verified in a variety of pharmaceuticals, including ranitidine and metformin. The present technical understanding of the various NDSRIs, impurity generation, risk factors, reaction conditions, analytical methods for detection, and potential mitigation strategies will all be covered in this study. We will explore the enormous gaps in mechanistic knowledge that still exist while also highlighting the scientific advancements that have been made in this area of study, which is currently under development.

KEYWORDS: *N-nitrosamine, Sartans, Ranitidine, Metformin, Mutagenic impurities, Carcinogenicity.*

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INTRODUCTION:

Nitrosamines are a group of organic compounds containing the Nitroso functional group. They can be found in water, food, tobacco, pesticides, or plastics, but received public attention in mid-2018, when they were also found in medicinal products (1). Among them, N-nitrosamines are so potent mutagenic carcinogens that they are referred to as the "cohort of concern" by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (2). ICH M7 (R1) classifies Nitrosamine impurities as Class 1 Genotoxic impurities, which is known to be mutagenic and carcinogenic, on the basis of both rodent carcinogenicity and mutagenicity data (4).

Nitrosamines have been classified based on their carcinogenic potential into four groups by the International Agency for Research on Cancer (IARC), World Health Organization (WHO) (1987). Group 1 compounds, e.g., N-nitrosonornicotine (NNN) and 4-(methylnitrosoamine)-1-(3-pyridyl)-1-butanone (NNK), have sufficient incidences of carcinogenic effects on humans while Group 2A compounds such as N-nitroso-dimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA) are probably carcinogenic to humans with limited evidence in humans but sufficient evidence in animals. Examples of Group 2B such as 1-methyl-4-nitrosopiperazine (NMP) and 1-cyclopentyl-4-nitrosopiperazine (CPNP) are considered to be possibly carcinogenic to humans with limited evidence in humans as well as in animals. The nitrosamines, with inadequate data on their carcinogenicity in human and experimental animals, are classified in group 3 such as N-nitroso-diphenylamine and N-nitrosoguvacoline (3). Nitrosamine impurities produce effect on the genetic material by means of mutations through chromosomal breaks, rearrangements, covalent binding or insertion into the DNA during replication. These changes within the genetic materials produce by the exposure to very low levels of Nitrosamine impurities can result in cancer (5).

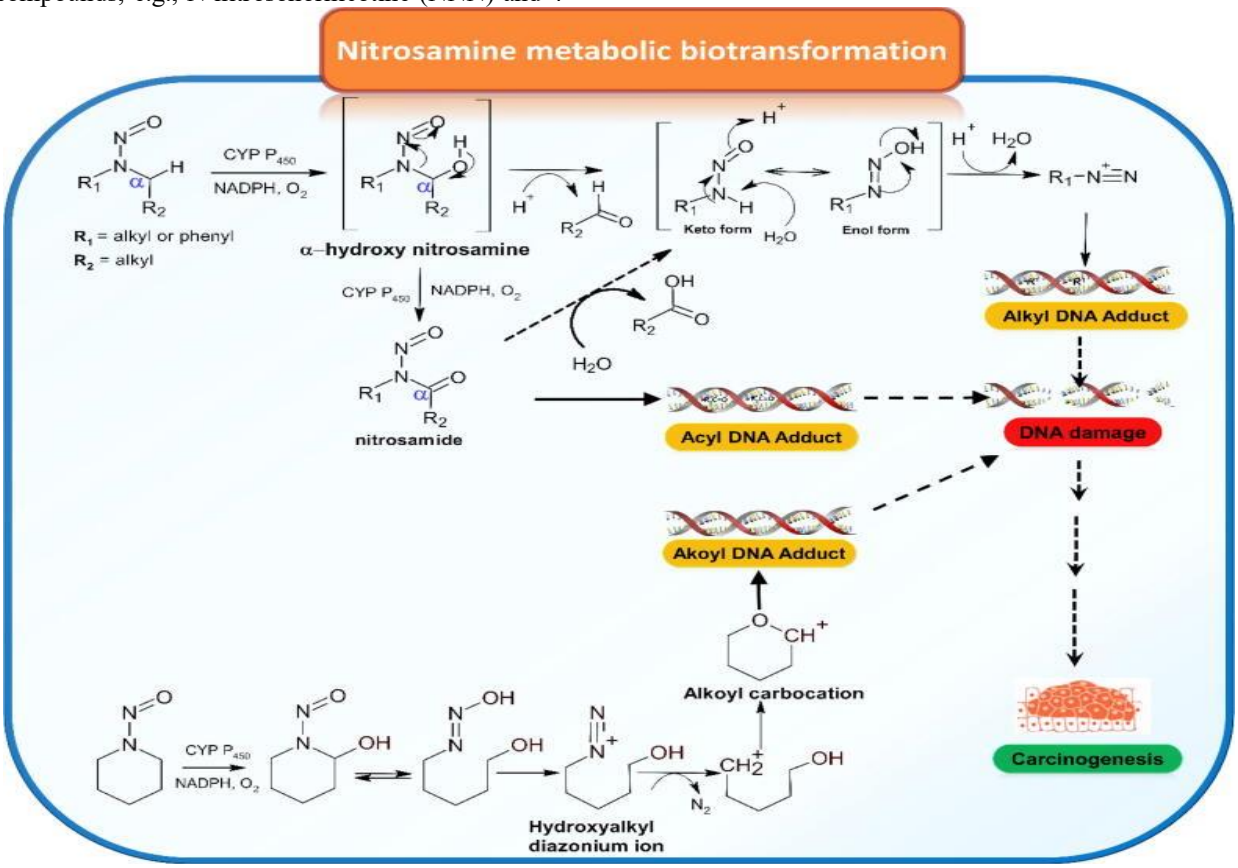

Fig 1. Nitrosamine metabolic biotransformation pathways.

A total of 10 nitrosamine impurities is reported for mutagenic potential.
1. N-Nitroso-dimethylamine
2. N-Nitroso-diethylamine
3. N-Nitroso-methyl ethylamine
4. N-Nitroso-di-n-propylamine
5. N-Nitroso-di-isopropyl amine
6. N-Nitroso-di-n-butylamine
7. N-Nitroso-diphenylamine
8. N-Nitroso-pyrrolidine
9. N-nitroso-piperidine
10. N-Nitroso-morpholine

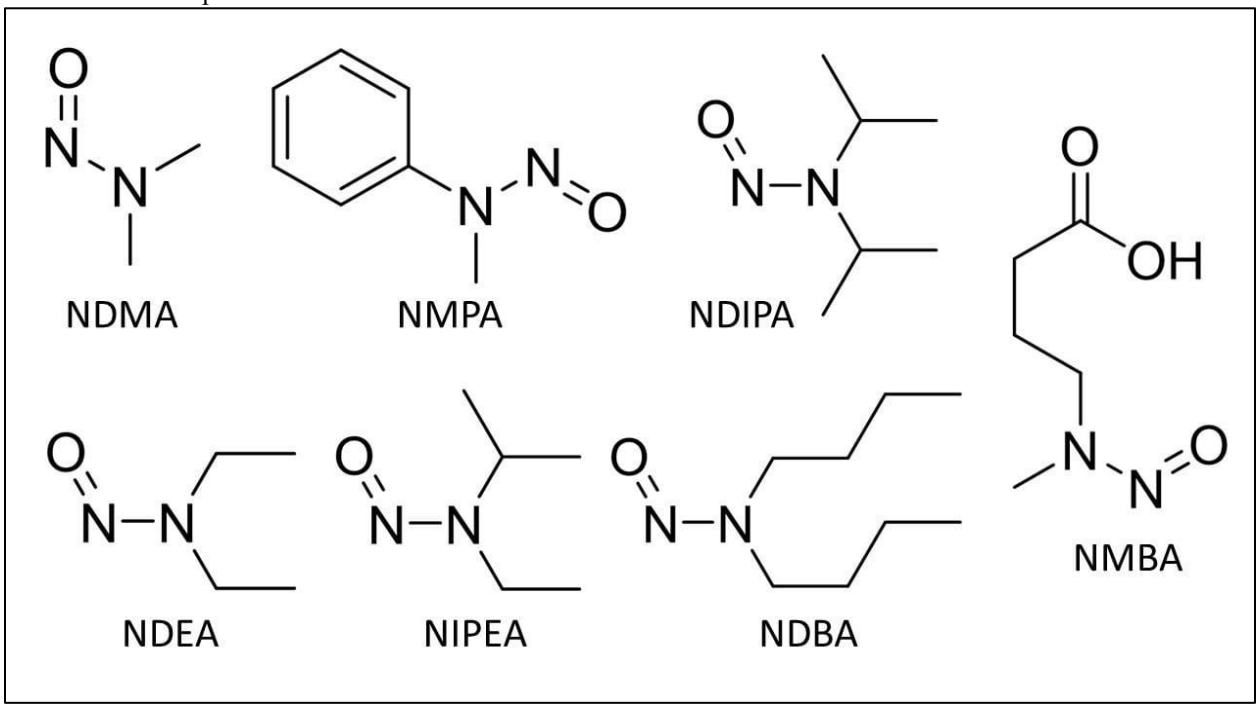

Fig 2. Structures of Nitrosamines Impurities

FORMATION AND SOURCES OF NITROSOAMINE IMPURITIES [9]:

Nitrosamine impurities can get incorporated into the drug substance and drug product basically through process formation, direct introduction, degradation or cross-contamination. Manufacturing of drug substances involves raw material, intermediates, solvents, chemicals and reagents [6-8]. Through these stages, if this impurity is formed or

present it may get incorporated and carried forwarded to drug product as shown in fig. 3.

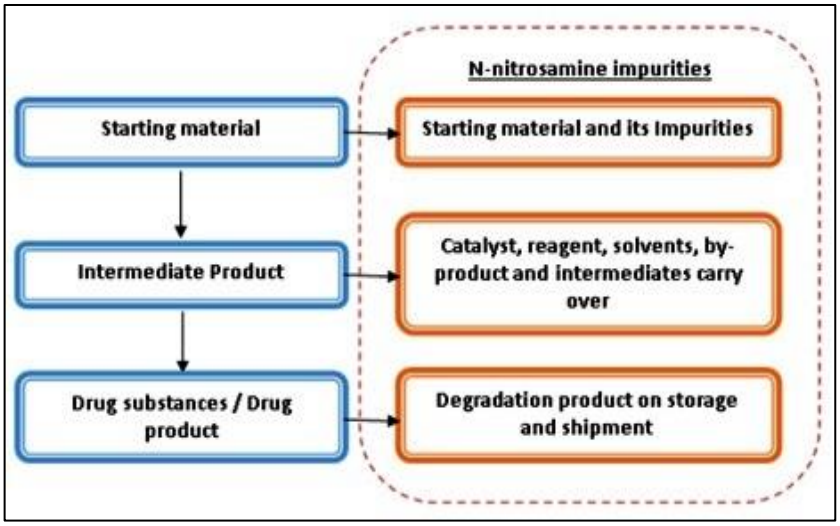

Fig 3. Sources of Nitrosamine Impurities

Formation of Nitrosamine impurity can occur when the use of sodium nitrite (NaNO2), or other nitro-sating agents, in the presence of secondary or tertiary amines within the identical or different steps of the manufacturing process. By Using sodium nitrite (NaNO2), or other nitrosating agents, in combination with catalysts, reagents, and solvents (e.g., DMAc, DMF, and NMP), which are vulnerable to degradation to secondary or tertiary amines, within the identical or different process steps. Formation of Nitrosamine impurity can occur in presence of degraded raw materials within the API manufacturing process (e.g., solvents, reagents and catalysts). Use of contaminated recovered material and contaminated recycled materials (e.g., solvents, reagents and catalysts), including recovery outsourced to 3rd parties who are do not seem to be tuned in to content of the materials they are processing and recovery processes carried out in non-dedicated equipment. Carry-over of nitrosamines intentionally generated (e.g., as intermediates) during the manufacturing process. Formation of Nitrosamine impurity can occur when the use of sodium nitrite (NaNO2), or other nitrosating agents, in the presence of secondary or tertiary amines within the identical or different steps of the manufacturing process.

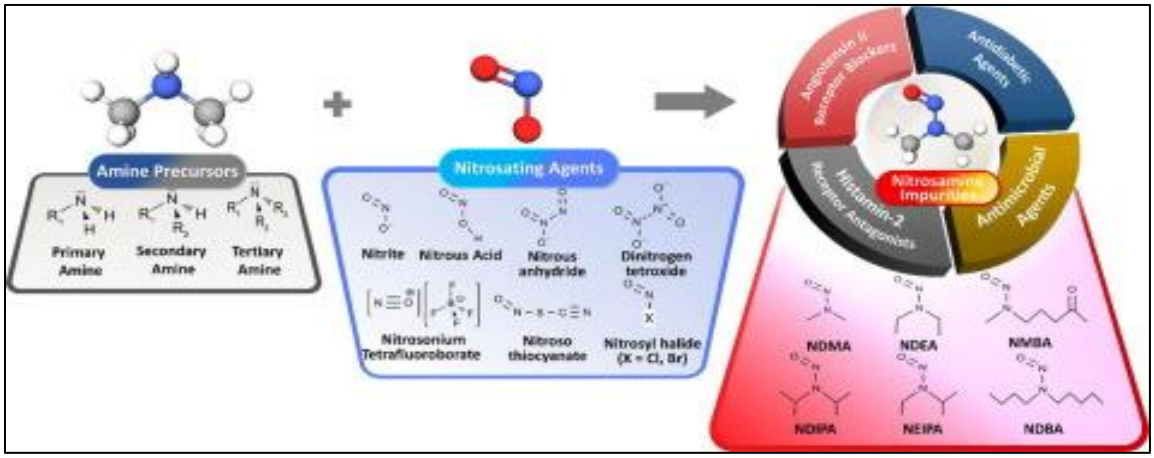

Fig 4. Formation of Nitrosamine Impurities

CHEMISTRY OF NITROSAMINES [10-12,20-22]

The chemistry of nitrosamine formation is very complex. Low molecular weight nitrosamines can generally be isolated either as distillable liquids or crystalline solids. Due to the large dipole moment of the N-NO group and depending on lipophilicity of the attached substituents, they are partially soluble in aqueous media and readily soluble in organic solvents. This solubility characteristic has important implications when considering the efficiency of their purge from reaction mixtures by standard aqueous work-up procedures and in preparation of analytical samples. In the case of high molecular weight

nitrosamines, such as APIs nitrosated on a secondary amine (known as a nitrosamine drug substance related impurity, or NDSRIs), other partial structures and embedded functional groups in these molecules can modify physicochemical properties significantly, which prevents any general conclusions for this sub-class of nitrosamines being drawn. The properties and reactivity of nitrosamines are heavily influenced by the zwitterionic resonance structure of the N-NO bond, which has significant double bond character resulting in planarity that hinders rotation. The N-NO bond can be broken thermally but typically requires exposure to high temperatures (400-500°C). Nitrosamines are also

susceptible to photolytic degradation on exposure to light, enabling the application of UV photolysis as a nitrosamine removal technique from water. Formation of N-nitrosamines are done by the reactions of organic amines and their derivatives with nitrosating compounds; however, the most stable nitrosamines are formed from secondary amines. Generally, Amines are categories as primary, secondary, and tertiary amine. Nitrosating agents can react with Primary amines and to generate highly reactive, unstable diazonium ions, which continually decompose to release molecular nitrogen. It's also feasible for the occurring diazonium ion to reacting with the starting primary amine to formation of a secondary amine, which might then undergo nitrosamine formation. In the case of a molecule with two primary amines that are segregated by 4 to 5 carbons, a cyclic nitrosamine can form. However, indirect nitrosation of primary amines is low producing because of the instability of the diazonium ion and the requirement for two continuous reactions to take place.

Secondary amines are the foremost likely amines to react and formation of nitrosamines occur, however the rate of reaction is dependent on the both reactivity and concentration of starting materials. A characteristic chemical reaction procedure for secondary amines to formation of nitrosamines is provided in figure.

Tertiary amines are not directly reacting with nitrosating agents, but they can primary undergo nitrosative detachment to secondary amines, which may then after form nitrosamines. While this can be chemically feasible, the reaction is slow and generally requires more amount of the nitrosating agent and high temperatures. It is critical to note, that the tertiary amines (such as generally used diisopropylethylamine and triethylamine) can contain secondary amines as impurities and/or can decompose into secondary amines that may then proceed to more readily form nitrosamines.

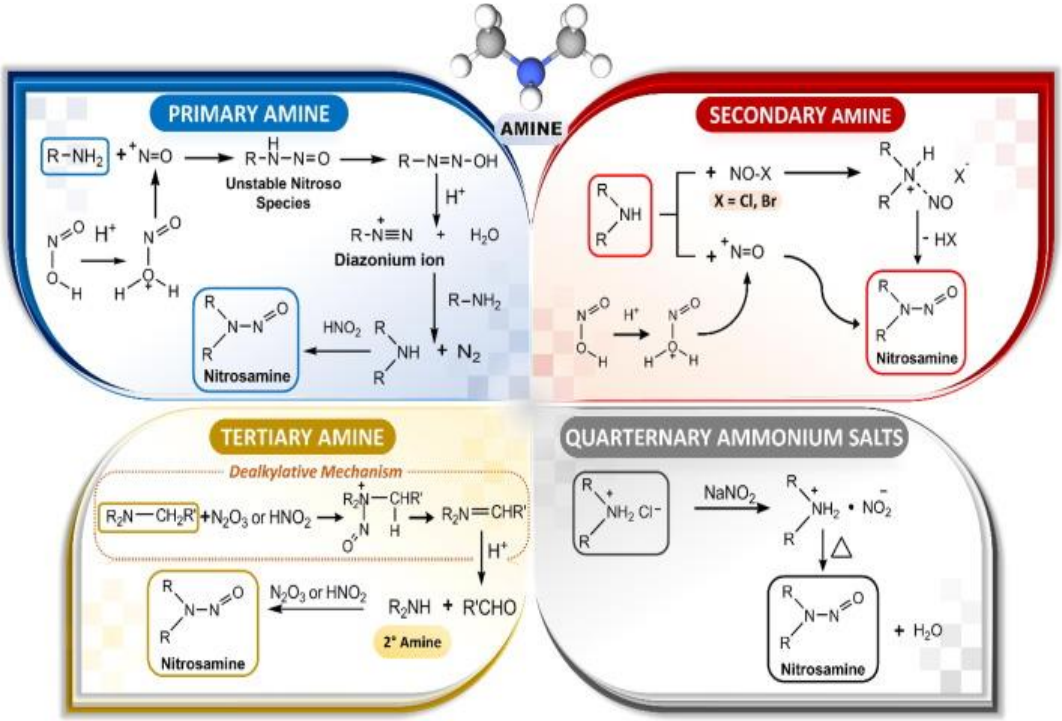

Fig 5. Pathways of nitrosamine formation between nitrosating agents and primary, secondary, and tertiary amines and ammonium salts

ROOT CAUSE IDENTIFICATION AND ITS PREVENTION:

Possible root causes of nitrosamine contamination in pharmaceuticals are either from API or from Manufacturing process.

ARBs

With the exception of eprosartan, the core structure of ARBs or sartans is a biphenyl functional group. Structurally, ARBs are divided into two categories: tetrazole analogues and non-tetrazole analogues. Nitrosamines presenting in ARBs are generally formed during API manufacturing and are identified as in-process impurities. Under the preferred condition of the nitrosation reaction, co-existing primary, secondary, and tertiary amines or quaternary ammonium salts react with nitrosating agents to generate nitrosamine impurities during the synthesis of ARBs. The chemical structures of ARBs are unrelated to those of the contaminating nitrosamines because the amine precursor arises from residual amines or amides in the organic solvents used in API synthesis. Nitrosating agents, including sodium nitrite, nitrous acid, nitrous anhydride, and nitrosyl halides, can arise from recycled solvents or reused catalysts from different processes or across manufacturing lines with inadequate control and inappropriate monitoring. Amines and nitrosating agents during the synthesis of ARBs can also arise from

1) impure starting materials or intermediates in the upstream step,

2) carry-over of other manufacturing processes along the same production line, and

3) cross-reaction between nitrocellulose in the packaging materials and some printing inks. ARBs containing a tetrazole ring such as valsartan, losartan, irbesartan, candesartan, and olmesartan are prone to nitrosamine contamination whereas non-tetrazole ARB analogues such as eprosartan, telmisartan, and azilsartan are unlikely to contain nitrosamine impurities.

Histamine-2 (H2) receptor antagonists

NDMA is a disinfection by-product of ozonation and chlorination that typically causes environmental problems. Therefore, its elimination has attracted the attention of chemists and environmentalists in many countries. Recently, NDMA was found in the wastewater treatment process of a ranitidine manufacturer, implying a relationship between NDMA and ranitidine, an H2-receptor antagonist that can potentially be an NDMA precursor. The risk of NDMA formation is related to the chemical structures of compounds containing functional groups for N-nitrosation. The nitro functional group and dimethylamine side chain found in ranitidine and nizatidine are probably implicated in NDMA generation. In contrast, cimetidine and famotidine are free from NDMA contamination. The possible causes of NDMA contamination in these compounds are contamination during the manufacturing process, the inherent instability of APIs, and degradation of drug impurities as summarized in fig 7.

Unlike NDMA contamination in ARBs, NDMA levels in ranitidine and nizatidine products significantly depend on storage time and temperature. It was reported that NDMA forms directly via ranitidine and is released much faster from ranitidine impurities. DMA is believed to be generated from ranitidine and its impurities containing dimethylamino and/or nitro functional groups. Whereas in vitro experiments have indicated a strong connection between ranitidine and NDMA formation, in vivo clinical studies in animals and humans have highlighted the potential carcinogenicity of ranitidine. The genotoxic effects of ranitidine have been studied in rodents since 1983. Experiments have demonstrated that ranitidine induces DNA fragmentation in the liver or mucosa in the presence of sodium nitrite. In a clinical trial, NDMA was found in urinary excretions after oral intake of ranitidine, suggesting that long-term administration of ranitidine or its related structures increases the cancer risk. For instance, McGwin (2020) reported that proportionate reporting ratios (PPRs) of the significant and elevated malignancy observation on pharyngeal, oesophageal, stomach, colorectal, hepatic, and pancreatic cancers were 9.24, 3.56, 1.48, 16.31, 2.64, and 2.18, respectively. The study results support the hypothesis that NDMA-contaminated ranitidine can increase the epidemiologic cancer risk.

Antidiabetic agents

Metformin, a dimethyl guanidine analogue, is structurally classified as a guanidine derivative. Unlike ranitidine, metformin lacks a nitro functional group in its chemical structure and requires an exogenous nitrosating agent for nitrosamine formation. It was reported that incomplete oxy-cracked metformin could carry several amine by-products, including NDMA, DMF, N-N-dimethylurea, dimethyl guanidine, and hydroxy acetonitrile. The root cause of NDMA contamination in metformin differs from that in ARBs and histamine-2 receptor antagonists. Metformin is relatively stable and requires critical co-existing external factors such as nitrosating agents, moisture, and heat for conversion to NDMA. NDMA is generated during the manufacturing process of metformin drug products. Nitrites and nitrates available in pharmaceutical

excipients, such as CMC sodium, HPMC E5, HPMC K15M, and PolyoxTM are essential factors of nitrosamine contamination. Additional moisture in wet granulation and excessive heat during the drying process initiate nitrosation reactions in the manufacturing process of metformin. The NDMA contamination risk can be reduced by avoiding nitrite or nitrate-containing excipients or the wet granulation process. NDMA contamination in metformin can be sourced from the reaction between dimethylamine and the nitrosating agent during the API manufacturing process, the reaction between APIs and nitrosating agents from excipients under the wet granulation process, and degradation of APIs under very high temperatures. These possible source mechanisms are presented in Fig. 8. Another blood-sugar-lowering substance, pioglitazone, has also been cautioned by EMA for NDMA contamination. As preliminarily reported by the manufacturer, NDMA in pioglitazone is sourced from sodium nitrite and hydrobromic acid found in an early step (before the use of DMF) and hydrochloride in a later step.

Antimicrobial agents

In August 2020, the FDA noted two nitrosamine impurities in the antituberculosis agents Rifampin and Rifapentine. The contaminated nitrosamines are structurally related to the APIs, implying that two nitrosamine analogues (MNP and CPNP) originate in the drug synthesis process. CPNP is thought to arise from an intermediate step in rifapentine production. The root cause of MNP formation remains unidentified by manufacturers but probably originates similarly to rifapentine contamination. The nitrosamine levels in many rifampin and rifapentine products in the US exceed the AI interim limits (0.16 ppm for MNP in rifampin and 0.1 ppm for CPNP in rifapentine). As summarized in Fig. 9, MNP and CPNP contamination in rifampin and rifapentine can occur when nitrosating agents introduced during the manufacturing step react with 1-methyl piperazine and 1-cyclopentyl piperazine, respectively.

Other medicines

Over two years of nitrosamine detection in ARBs, global health authorities and pharmaceutical industries have learned important lessons. Nitrosamines can contaminate not only tetrazole containing sartans, but also other tetrazole drugs such as Cefamandole, ceftezole, tedizolid, letrozole, and tomelukast with similar risk. This issue has dramatically impacted the quality and reliability of drug products, leading to a drug supply shortage. Moreover, to address patient safety concerns, some physicians and healthcare professionals have switched their therapeutic regimens. Although nitrosamine does not contaminate all medicines, a number of medications have been identified as nitrosamine precursors in waste water treatment. Concurrent formation of nitrosamines is favoured by the chloramine disinfection process. Regulators conducted a test of nitrosamine formation potential. They identified several APIs—ranitidine, nizatidine, carbinoxamine, chlorpheniramine, diphenhydramine, doxylamine, azithromycin, clarithromycin, erythromycin, roxithromycin, amitriptyline, diltiazem, metformin, escitalopram, sumatriptan, tramadol, tetracycline, and venlafaxine—with potential NDMA-conversion ability after treatment with chloramine disinfectants. In contrast, lidocaine is prone to NDEA contamination. Pretreatment of waste water with oxidizing agents can effectively minimize NDMA formation by adding lone-pair electrons to nitrogen via an ozonation pathway leading to N-oxide. Such findings imply that amine-based pharmaceuticals contribute significantly to nitrosamine conversion and, consequently, to pharmaceutical contamination. Risk evaluation and routine quality-control testing of pharmaceutical products are expected to place enormous burdens on both reference and generic drug manufacturers, who must deliver qualified, efficient, and safe medicines to patients.

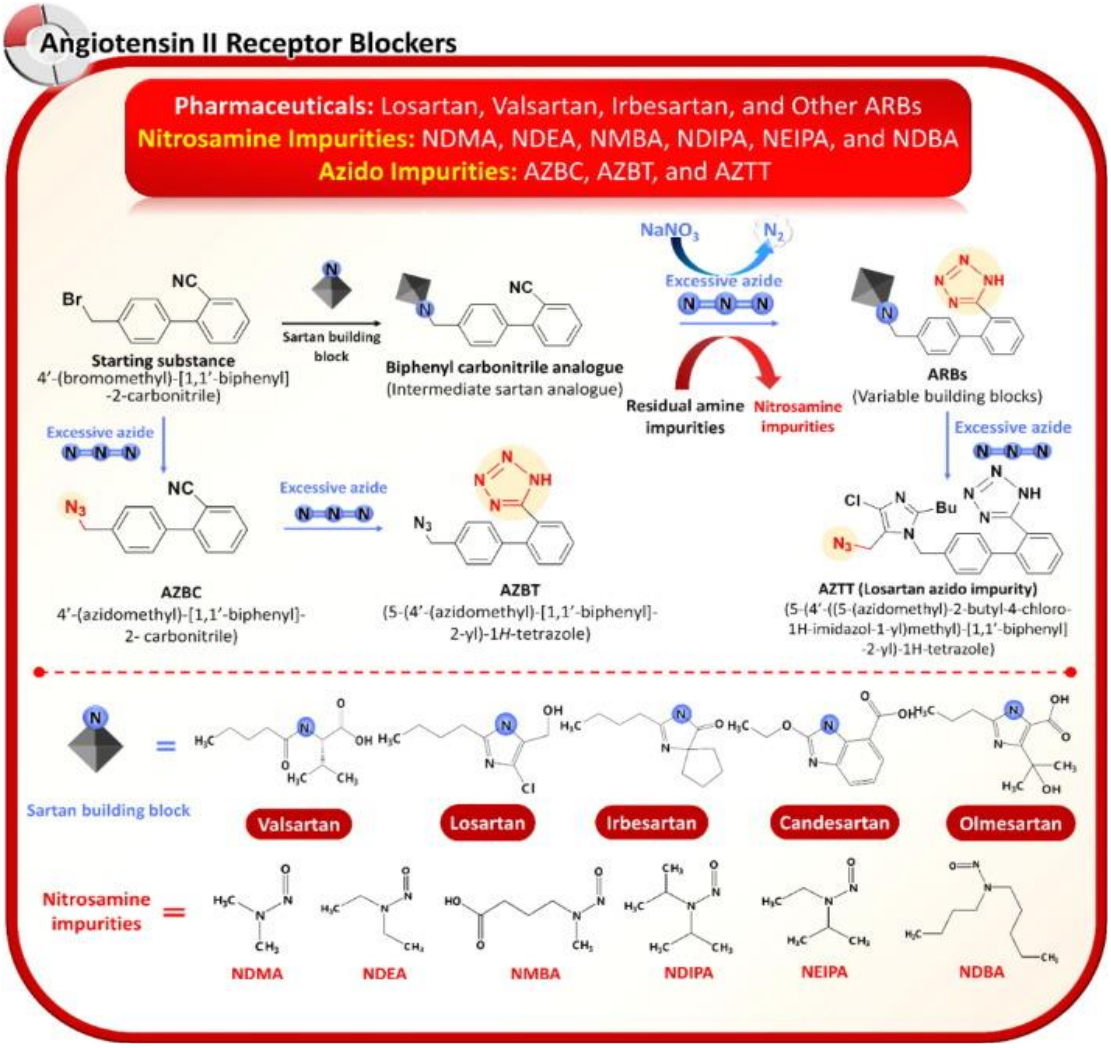

Fig 6. Possible root causes of nitrosamines and azido impurities in angiotensin II receptor blockers

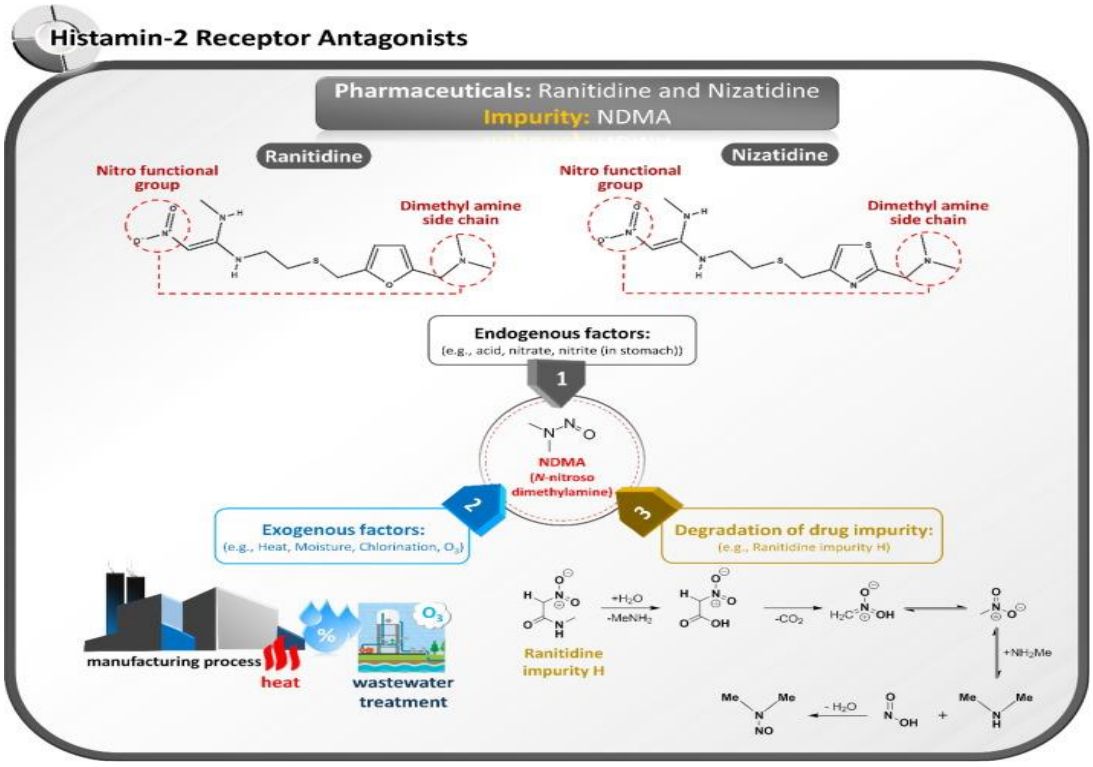

Fig 7. Possible root causes of N-nitroso dimethylamine (NDMA) contamination in ranitidine and nizatidine.

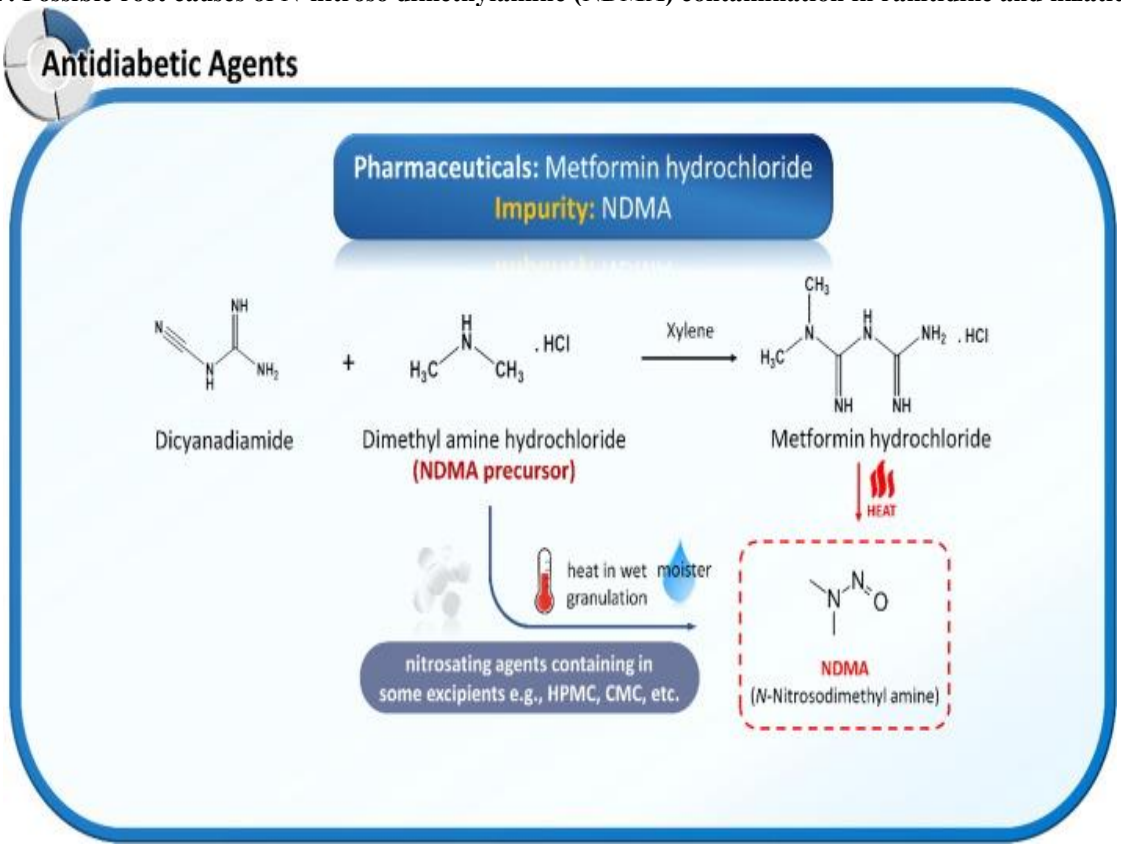

Fig 8 Possible root causes of N-nitroso dimethylamine (NDMA) contamination in metformin and its products.

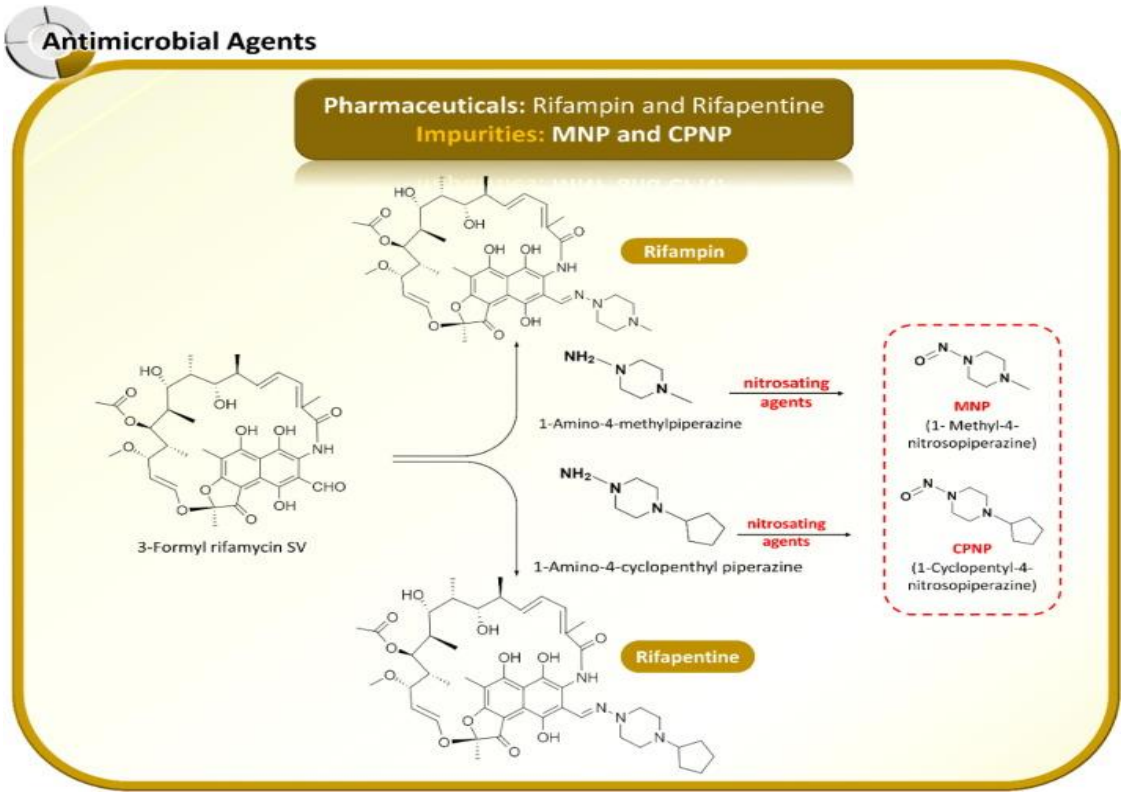

Fig 9. Possible root causes of 1-methyl-4-nitrosopiperazine (MNP) contamination in rifampin and 1-cyclopentyl-4-nitrosopiperazine (CPNP) contamination in rifapentine during the synthesis.

AVOIDING NITROSAMINE CONTAMINATION:

All amine along with nitrosating agents are considered to be antecedent in the generation of Nitrosamines impurities in the drug substance or drug products. Thus, the following precautions may lead to minimize these impurities in human medicinal products [13].

1. Nitrosamines impurities are formed when nitrites or other nitrosating agents react with the secondary or tertiary amine or quaternary ammonium salts are used within the same or different steps of the manufacturing process of drug substances. Thus, avoiding the use of these reagents can prevent Nitrosamine impurity formation.
2. Many of Nitrosamine impurities get purged out with the solvent. If these solvents are recovered and reused, there may be a chance of reintroduction of these impurities in the drug synthesis process.
   Therefore, use of recovered solvent should be avoided in the manufacturing process. Similarly, recovered catalyst may contaminate the drug with Nitrosamine impurities if reused.
3. Contaminated raw material, intermediate and reagents used in drug substance manufacturing are also the potential source of Nitrosamine impurities. The degradation product of raw materials and intermediate on storage in the presence of traces of nitrites may lead to the formation of Nitrosamine impurities. Hence, these materials should be properly stored and tested for Nitrosamine impurities.
4. Equipment used for the manufacturing of drugs substances may be cross contaminated with Nitrosamine impurities due to previous products. Equipment should be properly cleaned and checked for contamination.
5. The drug substances manufacturer should test and check the carryover of Nitrosamine impurities in various intermediate stages and if it is present, it should be controlled with the proper limit.
6. The manufacturer should modify the process to purge out amines, nitrites and Nitrosamine impurities at various stages. Control strategies should be implemented to detect and control Nitrosamine impurities in intermediate or drug substances.

Overall, it can be concluded that Nitrosamines in finished products can be very effectively controlled by selecting the synthesis path which minimizes the formation of these impurities and also observing implementing strict GMP requirements such as cleaning of equipment's and control of the recovery process for solvents may also lead to remove and limit the Nitrosamine impurities in drug substance and drug product.

USE OF NITRITE SCAVENGERS AND OTHER ADDITIVES TO PREVENT NDSRI FORMATION

An important recent development to control NDSRI formation is the addition of nitrite scavengers/nitrosation inhibitors to the formulation. While the inhibitory action of certain additives has been known for a long time and has been investigated in the context of food products (14), cosmetics and even medicines (15) to the best of our knowledge, the incorporation of specific nitrosation inhibitors in the formulation of pharmaceuticals has not yet been explicitly reported (although, as will be detailed in the following, many common excipients may serve as such). Based on literature reports, two main categories of additives can be identified to facilitate the control of (complex) N-nitrosamines in drug products:

(1) nitrite scavengers (anti-oxidants, amino acids, etc.) and (2) pH modulators (inorganic bases) Nitrosating agents (generally inorganic nitrite) can be chemically inactivated via a number of different strategies, namely redox reactions and quenching by nucleophiles. Natural antioxidants such as ascorbic acid or polyphenols are known to reduce nitrite (NO_2^-) to nitric oxide (NO), a gaseous small molecule no longer able to affect amine nitrosation; notably, NO is a well-known signalling molecule in the human body, involved in a number of biological processes (16). NO known to be captured by C-nucleophiles such as ferulic acid, caffeic acid and tocopherols (17-18). Typically, the primary products of these reactions are reactive species that undergo subsequent molecular rearrangements and fragmentations, leading to aldehydes, nitro-compounds and N−O-containing 5-membered and 6-membered heterocycles.

Another effective and well-known class of nitrite scavengers are primary amines which includes naturally occurring compounds such as amino acids. In this case, nitrosation leads to a short-lived diazonium salt that is trapped by nucleophiles in close proximity, for instance water. All proteinogenic amino acids undergo this reaction, most of them cleanly, affording nontoxic α-hydroxy acids as products (also known as the van Slyke reaction).62 The reaction goes through diazonium and αlactone intermediates. A similar process occurs in the case of aromatic amino acids, such as p-aminobenzoic acid (PABA); lactone formation cannot occur in this case and the final product is phenolic. Naturally occurring amino acids are endogenous substances and therefore present few safety concerns if used as excipients. The broad scope of this reaction provides flexibility in the choice of the most compatible scavenger, as will be exemplified later on in this section. Other amine derivatives (including ammonia) also undergo the van Slyke reaction; even less nucleophilic amines such as urea and sulfamic acid are known to scavenger nitrite, albeit with generally slower kinetics, unless the pH is below 2.67

ANALYTICAL METHODS FOR DETECTION OF NDSRIs [4,5,19]

The development of analytical methods is to identification of Nitrosamines impurities is the challenging task due to which the developed method can identified the genotoxic impurity at very less amount and below the TTC present within the complex matrices. Identification of nitrosamines in each drug is that the application of appropriate measurement technology focused on identifying very less amount (nanogram)of nitrosamines in solvents, intermediates, APIs and finished dosage forms. Regulatory agencies and pharmaceutical manufacturing firms around the world have developed and validated analytical method focused on nitrosamine detection. The developed analytical methods also required to be validated to conform to GMP requirements.

Several methods are published by the FDA to cover determining nitrosamine content in various active pharmaceutical ingredient (API)and finished product. The control strategy for nitrosamine impurity involves different process parameters like temperature, and extra purification, humidity, additional time cycles and such, during API manufacturing and similar stringent controls during formulation manufacturing. Most manufacturers have used LCMS systems to identified NDMA, to ensure that every batch is tested for NDMA before releasing the batch to the market so that the quality of the final product reaching patient is assured, so that recalls should not overly burden the healthcare system. The USFDA has suggest that the use of an LC-HRMS method for testing ranitidine due to lower temperature conditions method, higher temperature conditions of some test method may cause to generate NDMA. Most of the methods used for testing of Nitrosamines in drug substance and drug product utilize the chromatographic techniques such as

reversed-phase liquid chromatography (LC) or gas chromatography (GC) combined with various detectors such as mass spectrometry (MS), Ultraviolet spectrophotometry (UV) or nitrogen chemiluminescence (NCD) etc.

HPLC (high-pressure liquid chromatography)

Liquid chromatography is a very important separation technique that has a considerable impact in the area of pharmaceuticals and chemistry. HPLC consists of following parts that include the solvent reservoirs, degasser, low- or high- pressure gradient pump, guard column, sampling port, main column, detector, and computer display. Different stationary phases such as C18, SuperC18, C8, C5, C4, C4- 300, phenyl hexyl, HILIC, PFP, CSH, DAB, RP-Amide, SCX, CN-300, normal silica is available in the market for good selectivity and sensitivity.C18/Phenylhexyl are the most remarkable used for nitrosamine impurities detection. Initially, NDMA detection using diode array detector (DAD) in the wavelength range 230–233 nm was reported. In recently, NDMA identification using UV detector at 228 nm in valsartan drug was reported by French National Agency for Medicines and Health Products Safety, and Official Medicines Control Laboratories (OMCLs) of the General European OMCL Network (GEON). It is mentioning that reproducible analysis of nitrosamine using HPLC should be achieved by post-column photolysis and chemiluminescence detector (LC-PR-CLD). Reported the NDMA detection limit in valsartan drug 0.0085µg/mL and quantification limit 0.0285 µg/mL, respectively.

- **LC-MS/MS**

LC-MS is sophisticated technique, which separates and quantify the components from a complex mixture with the help of mass spectrometer. Mass spectrometer separates and identified the charged components. LC-MS is a technique that use to analyse large, ionic, polar, non-volatile, and unstable organic compounds. LCMS/MS analysis can be done via soft ionization and impurity analysis using various types of modern ionization sources including electrospray ionization (ESI) and matrix- assisted laser desorption ionization (MALDI), APCI, APPI, CI, EI, FAB, SIM5, Z Spray, and TSP.ESI and APCI are widely used for analysis of nitrosamine impurities present in various samples. several methods are being developed and reported for routine QAQC for nitrosamine impurities using LC-MS/MS.

- **GC-MS, GC-MS/MS, GC-MS-Head space, and GC-QTO**

GC-MS is a destructive and hard ionization technique for a qualitative and quantitative estimation of volatile organic compound or APIs. Although GC with various detectors can be used for nitrosamine detection, however, nitrogen, phosphorous detector (NPD) and nitrogen chemiluminescence detector (NCD) are the most suitable for the nitrosamine detection. On 24th September 2018 FDA has released a gas chromatography-mass spectrometry (GC/MS) headspace method for manufacturers and regulators to detect and quantify NDMA in valsartan API and finished drug products. As per that NDMA impurity should be less than 0.3 ppm. Various methods reported for identify/quantify nitrosamine impurities in sartans, and ranitidine using GC-MS, GC-MS/MS, GCMS-Head space, and GC- QTOF. USFDA quantified/ detected the presence of four toxic nitrosamine impurities, namely NDMA, NDEA, NEIPA, and NDIPA in valsartan drug using GC-MS/MS-Head space (HS) technique.

ANALYTICAL METHOD VALIDATION [4]

A general definition of validation is establishing documented evidence which provides a high degree of assurance that a specific procedure, process, equipment, activity or system will consistently produce a product meeting its predetermined specifications and quality attributes. Validation is an important characteristic after the development of any analytical method because it is closely related to the quality of the results of product. All analytical methods, whether qualitative or quantitative are necessary to be validated. The degree of validation is various for the type of method and its application. Validation is an essential activity in the process of impurities profiling where the developed analytical method used for the detection of genotoxic impurities in drug substances is validated in order to establish that the method is suitable for its aimed purpose. The analytical methods are validated with accuracy, precision, linearity specificity, ruggedness, robustness and forced degradation parameters in accordance with ICH Harmonized Tripartite Guidelines.

RISK ASSESSMENT AND LIMITS FOR NITROSAMINES

At present, generally there are insufficient data on carcinogenic potency of nitrosamines in humans, although most substances were found to be carcinogenic in vivo. The potential risk for humans is therefore extrapolated from animal data and varies by orders of magnitudes for different nitrosamines. As a precaution, conservative methods are employed to set limits for mutagenic carcinogens. Existing

pharmaceutical guidance [ICH M7 (R1)] (7) covers mutagenic carcinogens and applies a threshold of toxicological concern (TTC) approach with defining a negligible risk level (a theoretical 10−5 excess lifetime risk of cancer) to set an acceptable intake (AI) for any mutagenic substance. The methods upon which the TTC is based are generally considered to be very conservative since they involve a simple linear extrapolation from the dose giving a 50% tumour incidence (TD50) to a 10−5 incidence, using TD50 data for the most sensitive species and most sensitive site of tumour induction. For most mutagenic chemicals, a generic TTC of 1.5 µg/day can be justified, however it excludes structural groups of such high potency, for which intakes even below the TTC of 1.5 µg/day potentially could be associated with a carcinogenic risk exceeding the 1 in 105 incidences. Such chemicals are referred to as the "cohort of concern" and nitrosamines belong to this group. For nitrosamines, ICH M7(R1) recommends calculating a substance-specific AI using the TD50 or Benchmark Dose Limit (BMDL10) of animal carcinogenicity studies performed with the specific nitrosamine as "points of departure," i.e., starting points for the calculations.

Linear extrapolation of cancer risk from animals to humans using Haber's law is a conservative approach agreed by human medicines committee (CHMP) of the EMA for setting limits for nitrosamines in pharmaceuticals based on life-time exposure to the impurity. However, ICH M7(R1) also allows upward adjustment of impurity levels in products intended for less than lifetime (LTL) use based on the linearity paradigm "dose × time = constant." If this concept were applied to nitrosamines, this would result in an acceptance of much higher levels, especially in medicinal products used only short-term.

Therefore, the default approach for calculation of nitrosamine limits is based on the AI for lifetime exposure as per the principles of ICH M7(R1). However, there may be exceptional cases, such as critical medicinal products with limited therapeutic alternatives that would be at risk of shortage in case of recalls or disruption of supply if an AI for lifetime exposure is applied. In such cases, when a N-nitrosamine cannot be kept below the AI limit, higher limits may exceptionally be accepted by the relevant authorities, but only after having performed a benefit/risk evaluation, with the assessment being coordinated at EU level through the Scientific Committees of EMA to facilitate a harmonised approach across Member States.

Acceptable limits of nitrosamines

Since 2017, the ICH Guideline M7 (R1) has been established for the assessment and control of mutagenic impurities in pharmaceuticals. Nitrosamines are classified as Class-1 impurities as they are known mutagenic carcinogens. The ICH Guideline M7 (R1) is based on the "Threshold of Toxicological Concern" (TTC) concept, which defines the tolerable amounts of lifetime exposure with negligible human cancer risk. The TTC is theoretically associated with the potential of significant carcinogenic risk, stating that the cancer risk increases to one case in 100,000 (a theoretical excess lifetime cancer risk of 10^{-5}) when the acceptable limits of mutagenic impurities in drug substances and drug products are set at 1.5 ug/day.

Nitrosamine	AI Limit (ng/day)
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

FDA recommends the following acceptable intake (AI) limits for the nitrosamine impurities NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA. We further recommend that manufacturers use these AIs when determining limits for nitrosamine impurities in APIs and drug products.

These limits are applicable only if a drug product contains a single nitrosamine. If more than one of the nitrosamine impurities identified is detected and the total quantity of nitrosamine impurities exceeds 26.5 ng/day (the AI for the most potent nitrosamines) based on the maximum daily dose (MDD), the manufacturer should contact the Agency for evaluation. For drug products with an MDD of less than 880 mg/day, a recommended limit for total nitrosamines of 0.03 ppm is not more than 26.5 ng/day and is considered acceptable. For drug products with an MDD above 880 mg/day, the limit for total nitrosamines should be adjusted so as not to exceed the recommended limit of 26.5 ng/day.

Drug	Maximum daily dose (mg/day)	Acceptable Intake NDMA and NMBA (ng/day)	Acceptable intake NDMA and NMBA (ppm)	Acceptable intake NDEA (ppm)
Valsartan	320	96	0.3	0.083
Losartan	100(US)	96	0.96	0.27
	150(EMA)	96	0.64	0.177
Irbesartan	300	96	0.32	0.88
Azilsartan	80	96	1.2	0.33
Olmesartan	40	96	2.4	0.66
Eprosartan	800	96	0.12	0.033
Candesartan	32	96	3.0	0.83
Telmisartan	80	96	1.2	0.33
Metformin	3000	96	0.032	-
Rifampin	600	-	-	-

CONCLUSION:

Safety worries about nitrosamine impurities have spread throughout the pharmaceutical industry, including tetrazole-based medications and analogues of amines. Over the past two years, attention has turned from very potent small molecule N-nitrosamine impurities produced during drug substance manufacturing to NDSRIs, which are primarily produced during the formulation and storage of the therapeutic product. According to ICH M7 (R1) guidance or other quality regulations, pharmaceutical industries must assess the risks of chemical reagents or potential by-products that produce reactive genotoxic impurities such as azido and nitroso compounds, epoxides, hydrazine's, alkyl halide derivatives, etc. in the drug product registration dossier. Although there are well-established and documented methods for preventing or reducing the occurrence of N-nitrosamine impurities that result from the manufacture of medicinal substances, the management of NDSRIs in pharmaceutical products remains a serious concern. Impurities of highly mutagenic and carcinogenic Nitrosamine must be kept to a minimum in drug substances and drug products. We discuss numerous cutting-edge analytical techniques for accurately quantifying nitrosamines in complicated combinations. There are still a lot of gaps in our understanding of the mechanisms underlying the several risk factors that contribute to NDSRI generation in medicinal products, despite substantial advances in this area. Therefore, the control techniques for preventive and corrective measures depend on both a proper built-in concept of control limit criteria and a thorough grasp of the chemistry of nitrosamine synthesis and breakdown. Hopefully, increased cooperation and research will make it possible to reduce NDSRI levels appropriately, when necessary, allow for more precise risk assessments, and lessen the need for testing in the future. As scientific knowledge has grown, regulatory guidelines and policy have been periodically modified. As more information becomes available, additional advice changes can be anticipated.

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Disclosure of Conflict of Interest

The authors have no conflict of interest to declare.

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