Asian Journal of Chemistry; Vol. 35, No. 4 (2023), 794-804

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.27603

REVIEW

Nitrosamine Impurities: Assessing Concerns through Case Studies

MONALISHA JENA, MISBAH MUKADAM, DIVYA TELANGE, RUCHIKA DOLAS and C.S. RAMAA*

Bharati Vidyapeeth's College of Pharmacy (Affiliated to University of Mumbai), Sector-8, CBD Belapur, Navi Mumbai-400614, India

*Corresponding author: E-mail: ramaa.cs@bvcop.in

Received: 6 February 2023; *Accepted*: 7 March 2023; *Published online*: 30 March 2023; AJC-21175

N-nitrosamines are potential human carcinogens that can be ingested from a range of known sources, including food, drinking water, tobacco smoke and cosmetic goods. Remarkably, their prevalence in medicinal products went undetected until mid-2018. These contaminants were first detected in the active pharmaceutical ingredient (API) of valsartan and other sartan medicines were eventually implicated. The regulatory response to the nitrosamines issue included a recommendation to all marketing authorization holders (MAHs) for human medicinal products containing chemically synthesized active pharmaceutical ingredients to evaluate the potential hazards of nitrosamines in their products and implement appropriate risk mitigation checks and balances. The objective of this review is to investigate various realms associated with investigating how these genotoxic and carcinogenic impurities may be formed during the manufacture or preservation/storage of a wide range of drugs, including sartans (losartan, valsartan), anti-diabetics (metformin, pioglitazone) and a few antacids (ranitidine) and a thorough literature review on case-studies, drug-excipient interactions, metabolic activation and other prospects.

Keywords: Nitrosamines, Carcinogenicity, Nitrosation, Nitrosamine impurities.

INTRODUCTION

Nitrosamines, are a type of chemical molecule that contains a nitroso functional group linked to an amine (Fig. 1) and generally found in drinking water, beverages, tobacco smoke, cosmetics and foods such as cured and grilled meats, dairy products and vegetables [1]. Since the active substance valsartan (an antihypertensive agent that functions by preventing angiotensin II, a blood pressure-regulating hormone, from binding to its AT1 receptor) was discovered to be affected by *N*-nitrosodimethylamine (NDMA), the German Regulatory Agency (BfArM) and the European Medicines Agency (EMA) suspended the marketing authorizations for generic versions incorporating valsartan from Zhejiang Huahai Pharmaceutical (ZHP) in July 2018. Considering that it is exceedingly harmful, specifically to the liver, as well as being demonstrated in preclinical studies to be cancerous, the International Agency for Research on Cancer (IARC) ranks NDMA as 2A, asserting that it is most likely carcinogenic to humans [2]. As depicted in the reaction below (Fig. 2), nitrous acid is a precursor in the synthesis of nitrosating agents and then transformed to the active nitrosating species.

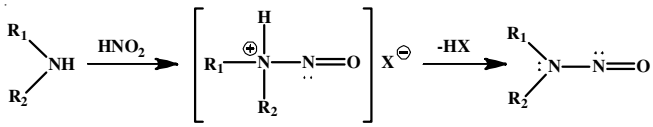

Fig. 1. General pathway depicting the formation of nitrosamines as a result of nitrosation of secondary amines

Nitrosamines necessitate metabolic activation to manifest mutagenic and carcinogenic impact as a consequence of their intrinsic stability at physiological pH. Cytochrome P450-dependent enzymes predominantly trigger metabolism by performing either a transient α-hydroxylation leading to transitory removal of an aldehyde or processive α-hydroxylations, which result in the generation of a nitrosamide. These processes generate extremely reactive carbocations or diazonium molecules capable of alkylating biological macromolecules such as DNA, RNA and so forth [3].

Considering escalating concerns about the nitrosamine contamination in medicines, nitrosamine production mechanisms and procedures for achieving meticulous control limits have been reported. Nitrosamines in medicaments can be traced back to the API production process, an interaction between

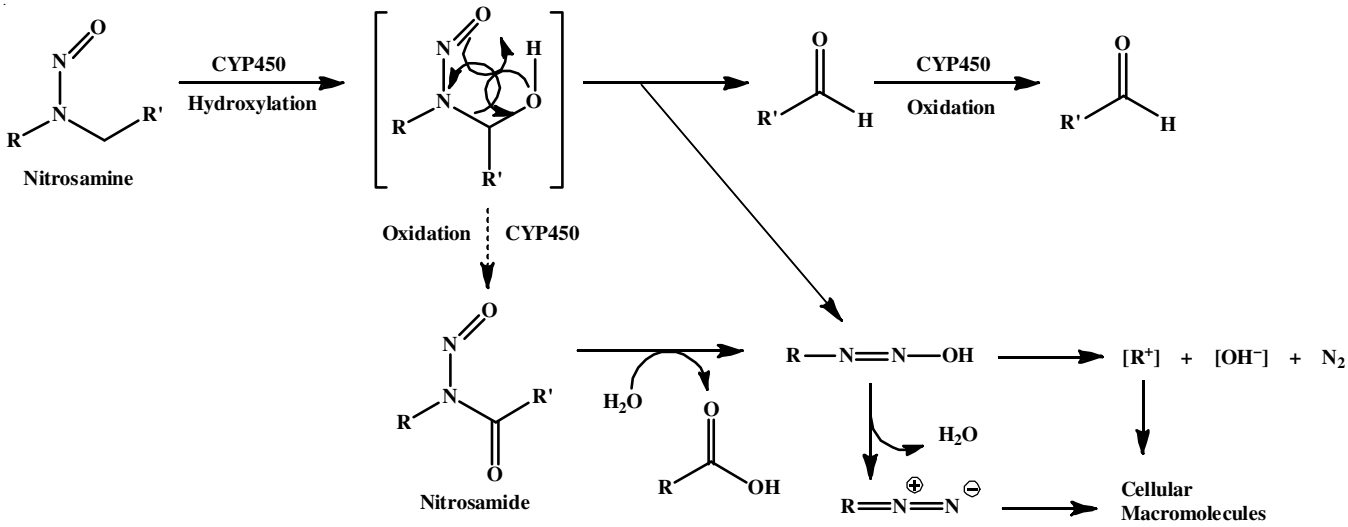

Fig. 2. Enzyme and non-enzyme mediated degradation of N-nitroso compounds

the API and reactants or poor storage settings [4]. Several drug recalls have happened given the existence of such nitrosamine drug substance related impurities (NDSRI's), this definitely needs a thorough strategy to prevent the formation of these impurities during synthesis and manufacture of the API and drug product as well as during packaging and storage [1]. To supplement other recent publications, we focus on the likely causes of nitrosamine production in this article. A systematic review of case studies specific to drugs which are used for long-term therapy in the pharmaceutical realm is emphasized.

Plausible sources of *N*-nitrosamine origination: Following the investigation of nitrosamine impurities in medicinal products in 2018, international regulators have required manufacturers and companies to assess the risk of nitrosamine contamination in their products [5]. Following the Article 31 complaint in regards to sartan medications, one API manufacturer alerted European Directorate for the Quality of Medicines (EDQM) that NDMA was confirmed in certain batches of their pioglitazone API, alongside valsartan from another API vendor. According to QWP and other advisors, a significant proportion of the causal factors serve as a reminder that repercussions can be massively diminished by rigorous computational modelling of design space, appropriate process innovation and sophisticated technological methods, which should be supported by appropriate *N*-nitrosamine standards in the final product to confirm that the medicinal product used by the individual is safe for consumption.

The European Medicines Agency (EMA) advised marketing authorization holders on how to examine prospective likelihood of aforementioned impurities in drug products. EMA warned MAHs in its wise counsel that it had unearthed an array of causal factors that opened the floodgates of nitrosamine emergence and contamination, which has been extensively discussed (Fig. 3).

Synthesis of APIs: Nitrosamine impurities have been reportedly produced amidst processing of active ingredients in the presence of specific raw materials and ancillary substances. EMA states that contaminants may not be entirely removed subsequently in the manufacturing process, despite subsequent

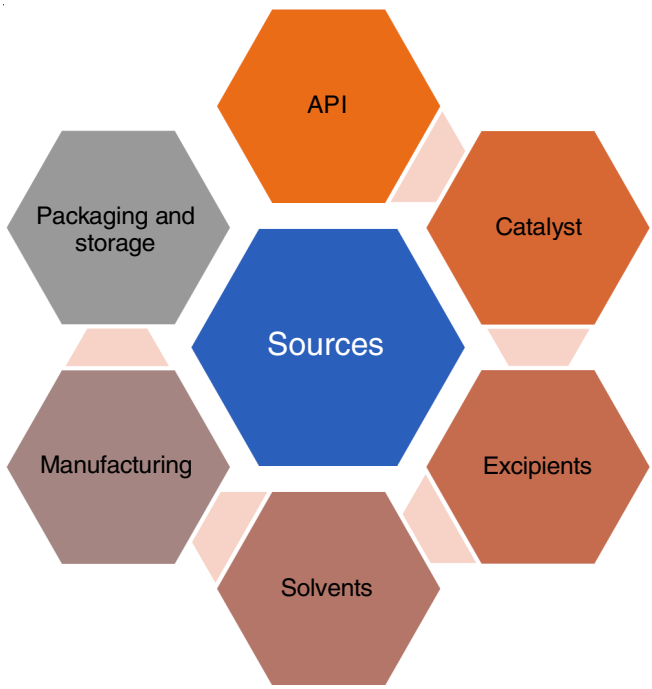

Fig. 3. Schematic diagram representing possible sources that pave the way for nitrosamine formation in pharmaceuticals

quality control steps. The incorporation of nitrites during API synthesis coupled with *sec./tert.*-amines may result in the formation of nitrosamine. *sec*-Amines are seldom found in the chemicals and reagents. Commonly used bases such as diisopropyl-ethylamine may contain *tert.*-amines. Nitrosamines might be generated if compromized starting ingredients, including intermediaries supplied by suppliers, are implemented. Some of the drug classes in which these impurities were found to be present upon recalls are sartans (losartan, valsartan), antidiabetics (pioglitazone, metformin), antacids (ranitidine) and a few antibiotics (doxycycline).

Catalysts: Catalysts namely triethylamine hydrochloride and others have been identified as *N*-nitrosamine precursors such as *N*-nitrosodiethylamine and *N*-nitrosodibutylamine.

NDEA formation (Fig. 4) was measured by scientists using a gas chromatographic technique for di- and trihydric phenols, in addition to various phenols of natural origin. Interestingly, a number of naturally occurring phenols have been demonstrated to lead to the emergence of NDEA under alkaline conditions, which can result in the ambiguous nitrosamine generation revealed upon analysis.

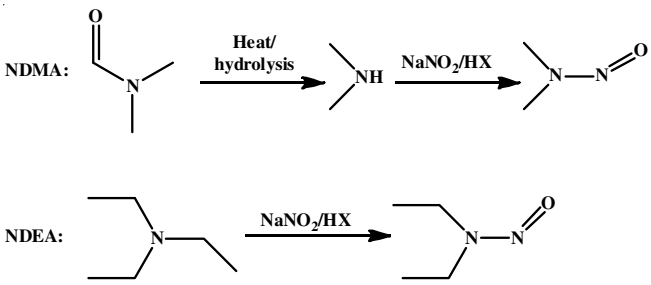

Fig. 4. N-nitrosamine generation and its route of formation

Excipients: Nitrates and nitrites are common nitrosating impurities found in most excipients in parts per million concentrations. Excipients that contain trace levels of nitrate or nitrite impurities include sodium starch glycolate (reported organic impurity was found to be 0-140 ppm), poly(vinyl pyrrolidone) and Lactose Fast Flo® [6].

For instance, Sluggett *et al.* [7] highlighted an unanticipated *N*-nitrosation reaction when propranolol-excipient blends were stored in a stability chamber with saturated sodium nitrite to regulate relative humidity. In this situation, it is hypothesized that *N*-nitrosamine derivatives are formed by nitric oxide evaporation from an aqueous nitrite solution towards the container's orifice, accompanied by diffusion into the solid drug or drug-excipient combination and subsequent interaction of NOx with *sec*.-amine. It is actively urged to adopt moisture controlled chambers or alternate salt solutions for humidity control during accelerated stability investigations of *sec*.-amine containing medicines.

Solvents: Solvents, reagents and catalysts such as dimethyl formamide, triethylamine, triethylaminehydrochloride and *N*,*N*-dimethylaniline (*N*,*N*-DMA) have been reported in literature to produce nitroso compounds when blended with sodium nitrite in the same step or sub-step of an API production process [8]. Nitrosamines may expected to be produced by hydrolytic and/or thermal degradation of solvents and subsequent N-nitrosation of the released *sec*.-amine [8].

Solvents namely dimethylformamide (DMF) and *N*-methylpyrrolidone (NMP) are the antecedants of amines likely to succumb to *N*-nitrosamine formation in the case of sartans [9]. Nitrosative dealkylation could lead to the formation of NDEA from the solvent/reagent triethylamine. Recently, NDMA was shown to be a disinfection side-product of chloramination and to a lesser extent, chlorination [10,11]. Anion-exchange water treatment can also result in the formation of NDMA [9].

Packaging materials: A novel underlying cause of NDMA/NDEA contamination of pharmaceutical products was recognized and reported to authorities in September 2019. The interaction of nitrocellulose present in lidding foil with printing ink resulted in the formation of *N*-nitrosamines, which were transmitted to finished piece through vaporization and liquifaction on the final product during the thermosealing blistering procedure [12]. According to Balser *et al.* [5], the deflagration temperature of plasticized nitrocellulose chips, for example, exceeds 180 ºC, causing the formation of various nitrogen oxides throughout thermally induced decomposition.

Axiomatically, the initiation of *N*-nitrosamines is linked to a pivotal chemical combination that previously considered to be legitimate, *i.e.* the concomitant inclusion of 2º or 3º amines in printing ink and nitrocellulose as a nitrosating agent in the lidding foil during printing [12]. Given that nitrocellulose is commonly used as primary packaging in the pharmaceutical domain, marketing authorization holders must scrutinize this causative factor for their medicinal products packaged in blisters, according to literature [1,13,14].

Storage: NDMA levels in ranitidine and nizatidine products are majorly influenced by storage duration and temperature, unlike NDMA pollution in sartan drugs. Based on the experimental findings by Abe *et al.* [15], it was concluded that ranitidine decomposes on heating and that NDMA is generated during headspace equilibration. Furthermore, the percentage of NDMA freshly generated during storage in the hermetically vacuum sealed vial to prevent moisture penetration, was significantly lower as compared to the closed and open ones. This suggests that exposure to the environment as well as high-temperature settings prompt the generation of NDMA (ranitidine in this case).

The aforementioned major determinants of nitrosamine infiltration may indeed originate within the same API production. As a result, several tactics may be required to uncover all probable sources of contamination. Analytical procedures employed to ascertain API integrity and established contaminants are unlikely to identify the existence of nitrosamine impurities. Furthermore, every source of failure may culminate in varied nitrosamine levels in different batches using the same process and API vendor, with contamination occurring in a few [16]. To delve greater depth into this subject matter, we have comprehensively presented case studies involving therapeutic drugs in which regulatory authorities acknowledged nitrosamine production and set interim limits for the acceptable intake.

Structural alerts: Prediction of nitrosamine impurity based on structure: Nitrosamines are contaminants formed as a result of preferential chemical moieties present in the analyte/API or reagent in a favourable environment such as pH of the reaction medium, dissociation constant of the molecule, acidity or basicity of the molecule [17]. The most susceptible molecules have been documented as those that contain *sec*.-amine (atenolol), *tert*.-amine (ranitidine), primary amide, *sec*.-amide, twisted *tert*.-amide and some heterocyclic compounds that do not contain amine as a functional moiety but contain nitrogen as a hetero-atom (Fig. 5).

Secondary amine: Nitrosamine formation in *sec*.-amine proceeds only when the amine is protonated; nitrosamine formation does not occur if the amine is not protonated [17]. Amine protonation is accomplished through a nucleophilic substitution process. As a result, the reactivity of *sec*.-nitrosamine

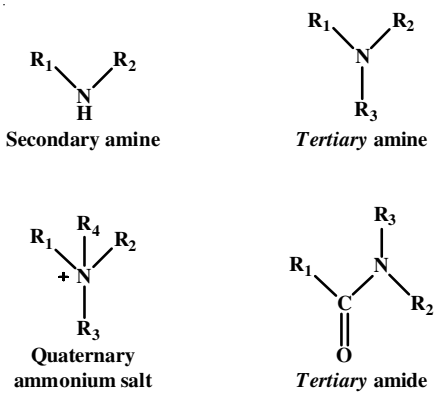

Fig. 5. Functionalities or "structural alerts" that pave the way for nitrosamine formation

will favour less basic amines; also, the basicity of the compound determines the rate of nitrosamine synthesis.

Tertiary amine: Aliphatic *tert.*-amine undergoes the nitrosation by nitrosative dealkylation, in which at least one proton at α-position to amine nitrogen activates further nitrosation *via* iminum intermediate. Nitrosamine synthesis also arises in cyclic *tert.*-amines *via* the ring opening mechanism [17,18].

Tertiary amine containing nitro group (*e.g.* ranitidine): Generally, *tert.*-amines are less reactive to the nitrosamine formation than *sec.*-amine, however, nitro group substitution within the molecule may result in the formation of nitrosamine under environmental impact [17]. Ranitidine is one such example, which is composed of both the above functionalities. Under the influence of environmental temperature, an intermolecular interaction occurs in which the nitro group from one molecule and the *tert.*-amine from another molecule combine, giving rise to NDMA.

Quaternary ammonium salts: Quaternary ammonium salts are not directly nitrosated; instead, they must undergo non-nitrosative dealkylation, which produces nitrosable *tert.*-amine, which is then nitrosated. As a result, quaternary ammonium salts are of the least concern [17,19].

Tertiary amides: *tert.*-Amide, like *tert.*-amine, does not immediately undergo nitrosation. They must first be hydrolyzed, which produces *sec.*-amine and carboxylic acid. In a subsequent procedure, the formed *sec.*-amine will easily undergo nitrosation, yielding nitrosamine impurity. For an extensive insight on the implications posed by structure/functionalities on the probability of nitrosamine formation, one may refer to the extensive study by Snodin [20], which indicates how *in silico* tools shall favourably be employed to predict the formation of nitrosamines, be it a structure-drawing program by Lhasa Ltd. or Loadscope (ICH M7 suite) to name a few.

Furthermore, Ponting *et al.* [21,22] proved that the carcinogenic potencies of *N*-nitroso compounds and nitrosamines as chemical classes had a log-normal distribution. Following further investigation, probable structural features were refined to generate a list of over 80 features encoded as SMARTS/SMILES.

The reactivity of nitrosamines is also a noteworthy aspect. There have hardly been a few studies concerning link involving the same alongwith carcinogenicity of such impurities. Reactivity, on the other hand, could be determined using a sensitive and quantitative approach, such as HR-MS [22]. Another segmentation strategy involves Bayesian probabilistic modelling, which pairs existing information with SARs and biomarker or metabolic data to provide a possibility to prediction; such a strategy might facilitate categorization [23].

Benzylic nitrosamines are somewhat more sterically inhibited than simple nitrosamines; this may be associated with enhanced potency because the benzylic position is particularly reactive due to conjugation with the aromatic system, which improves metabolism [24]. To add, benzylic nitrosamines, particularly *N*-nitroso-nornicotine, are commonly categorized as tobacco-specific nitrosamines.

Compounds containing carboxylic acids in any form are frequently toxic for reasons other than those surrounding the nitrosamine; rather, the acid changes the physico-chemistry and pharmacokinetics entirely [25-27]. For instance, carboxylic acid containing compounds are often firmly linked with plasma protein, that might lower the exposure and hence the ability for a high enough rate of mutation to overcome damage control and eventually trigger tumour growth. Additionally, since the substance is significantly more hydrophilic, the possibility of it being hydroxylated is diminished significantly [28,29]. This conjunction of enhanced plasma-protein association and greater clearance has been found to significantly reduce medication bioavailability and, as a result, efficacy.

Monitoring the impact of each structural attribute simultaneously and autonomously, whilst factoring in the combinations of features present and reducing the impact of multiple testing, can be utilized to garner statistically significant validation of SAR methodologies to the discrepancies in potency among various structural sub-groups of nitrosamines [30].

Case studies

Source: Raw materials during API manufacture

A. Reagent: Sartan drugs (Angiotensin II Receptor Blockers)

Valsartan, losartan, irbesartan and other sartan drug compounds are the category of angiotensin receptor blockers (ARBs), which alleviate elevated blood pressure and congestive heart failure [31]. They function by inhibiting angiotensin II that constricts blood vessels and increases blood pressure. They contains biphenyl tetrazoles, where the tetrazole ring necessitates the inclusion of azide reagents. To eliminate excess solvents, sodium nitrite is used, which produces nitric acid in an acidic medium. Nitrosamines are formed when the required solvent (DMF) reacts with the formed nitrous acid. The substitution of tributyl tin azide for sodium azide reduces the formation of nitroso impurities in valsartan (Fig. 6) [4]. Irbesartan, like valsartan, is an angiotensin receptor blocker with tetrazole ring in its structure. As a result of tetrazole ring synthesis, a nitrosamine impurity, NDEA, is formed.

Nitrosamine genesis was precipitated by the reaction of sodium nitrite as common NO_x with numerous *sec.*- and *tert.*-amine sources, as documented in the CHMP assessment report [8]. There are two distinct pathways for the production of *N*-nitrosamine [3], which are categorized as follows:

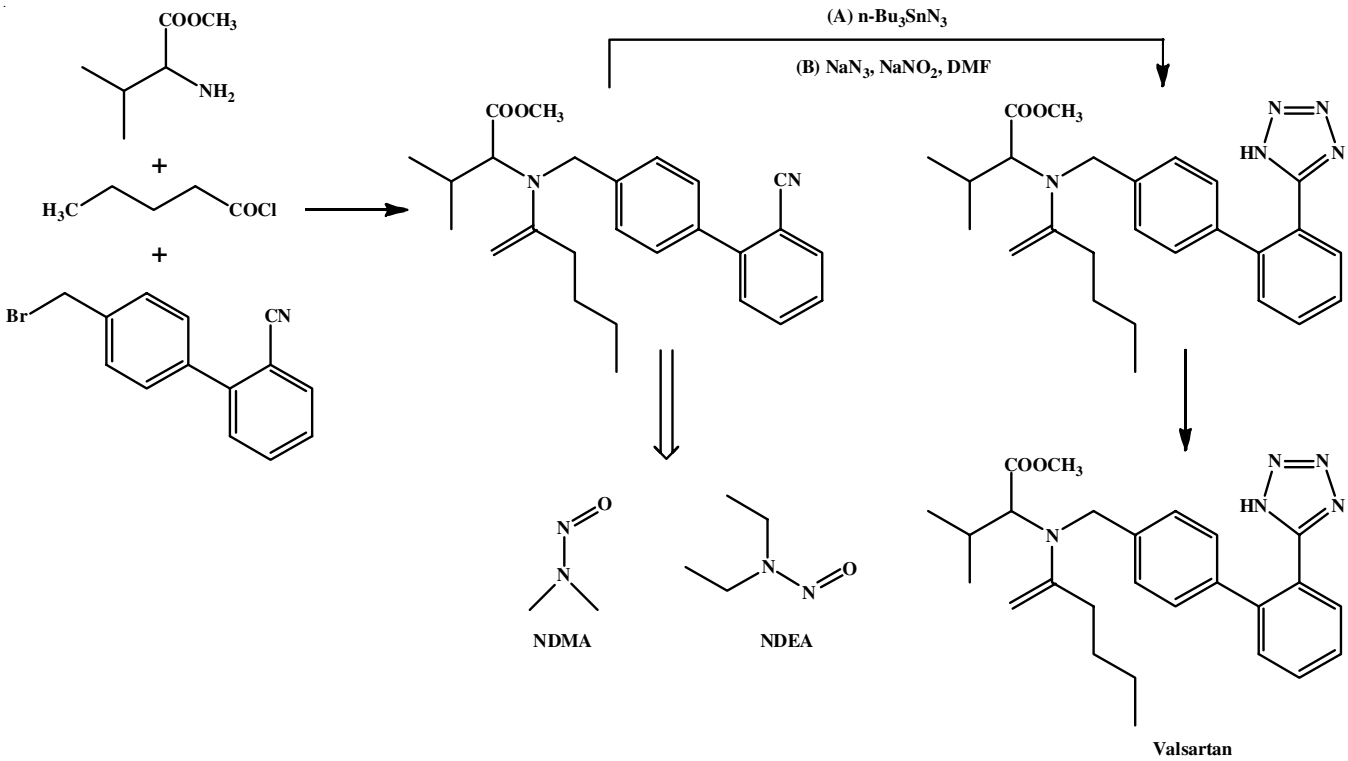

Fig. 6. Synthetic scheme for valsartan API

(i) Hydrolytic/thermal decomposition of the solvents DMF and NMP to generate DMA and MBA, followed by *N*-nitrosation to produce NDMA and NMBA.

(ii) *N*-Nitrosative dealkylation of trialkyl amines TEA, DIPEA and *N*,*N*-DMA, resulting in the formation of NDEA and NMPA; hydrolytic dissociation of TEA·HCl, resulting in TEA, followed by *N*-nitrosative dealkylation, resulting in NDEA.

B. Solvent

NDMA was found amongst certain pioglitazone hydrochloride (anti-diabetics) samples, the first case for a non-sartan medicament. Although NDMA remnants discovered were less than the established limit for this impurity (96 ng/day), no recalls of the affiliated pharmaceutical products were spearheaded. Regardless, the manufacturing routes of all pioglitazone sources in the EU were tested for *N*-nitrosamines.

Utilization of sodium nitrite and hydrogen bromide in an early step of the process, accompanied by DMF and HCl in a later step, was the preliminary root cause of nitrosamine formation (Fig. 7). This potential cause necessitates the administration of sodium nitrite or perhaps some nitrosating agent (NO_x) in several steps prior to the introduction of DMF. Use of NDMA-contaminated solvents (*e.g.* DMF) were also sought. It was determined that NDMA generation can be circumvented by substituting DMF as nitrosatable solvent with a non-nitrosatable solvent as modification methodology [5].

C. Reagents

Metformin (anti-diabetic agents): Metformin is an anti-diabetic medication utilized as a first-line oral glucose-lowering agent in the treatment of type 2 diabetes [32]. It diminishes the gluconeogenesis in liver, either directly or indirectly and increases glucose uptake, GLP-1 and modulates the flora in stomach. In late 2019, FDA discovered some extended-release metformin drug products containing unacceptable levels of NDMA [32-34].

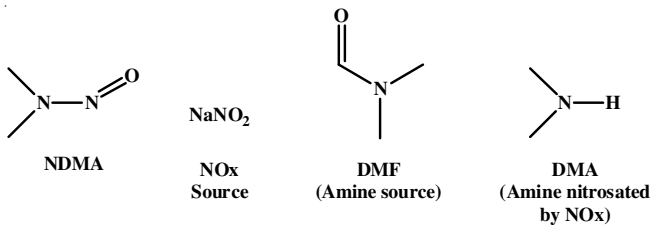

Fig. 7. Critical compound combination culminating in N-nitrosamine formation in pioglitazone HCl

Metformin is synthesized using the key reagents dimethyl amine and dicyanodiamide [35]. Dimethylamine remains in the final product and is used in the development of drug products. According to the European Pharmacopoeia, the limits of residual dimethyl amine in metformin monograph are 0.05% (500 ppm) in API. NDMA in metformin products may be attributed contamination during API production (because only a few batches/brands exceed the acceptable NDMA standards). Residual DMA is carried forward in the dosage formation process (Fig. 8), as amine precursor source in the synthesis/formation of N-nitroso dimethyl amine [1,31,33].

Rifampin (antibiotics): Rifampin, alternatively referred to as rifadin, rimactane and rifampicin, is perhaps the most effective antibiotic in clinical usage for the treatment of tuberculosis [14,32,34]. They come under the rifamycin class, particularly ansamycins, comprising a macrocyclic ring bridged spanning two non-adjacent positions of an aromatic nucleus.

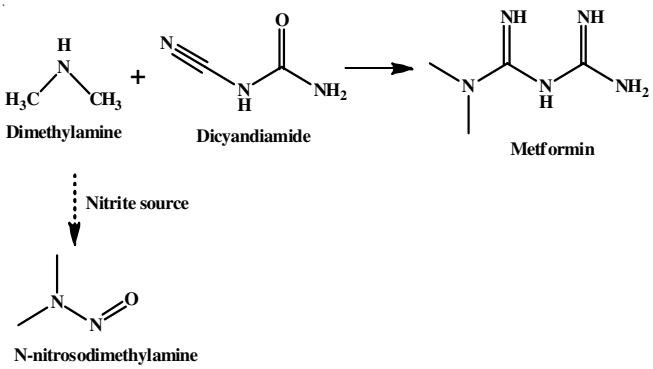

Fig. 8. Formation of nitrosamine impurity in metformin

Rifampicin is synthesized using rifamycin B; Mannich bases of rifamycin SV are formed by reacting formaldehyde with a lower alkyl, cycloalkyl or hydroxy-alkylamine. One may generate 3-formyl rifamycin SV, any of these Mannich bases is oxidized using a mild oxidizing agent such as alkyl nitrites (in this example, isoamyl nitrite). Subsequently, the reaction with 1-amino-4-methylpiperazine (AMP) produces rifampicin [14]. Alkyl nitrites can be hydrolyzed to produce free nitrites, which can then react with AMP to yield 4-methyl-1-nitroso-piperazine (MeNP) (Figs. 9 and 10). Atmospheric oxygen can oxidise hydrazine derivatives like AMP to nitrosamines, culminating in MeNP [36].

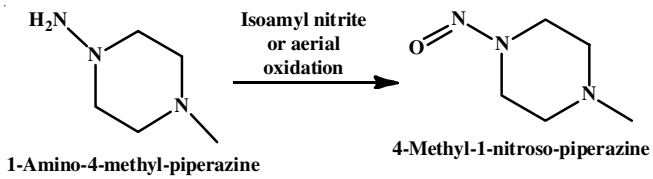

Fig. 9. Synthesis of 4-methyl-1-nitrosopiperazine-rifampicin

Rifapentine: Rifapentine is another antibiotic in the rifamycin class, which is prescribed to treat tuberculosis [14]. It inhibits the activity of DNA-dependent RNA polymerase in susceptible cells. The Food and Drugs Administration (FDA) received a grievance from a license holder in May 2020 stating that a specific batch of rifapentine drug component comprised 1-cyclopentyl-4-nitrosopiperazine (CPNP) [31].

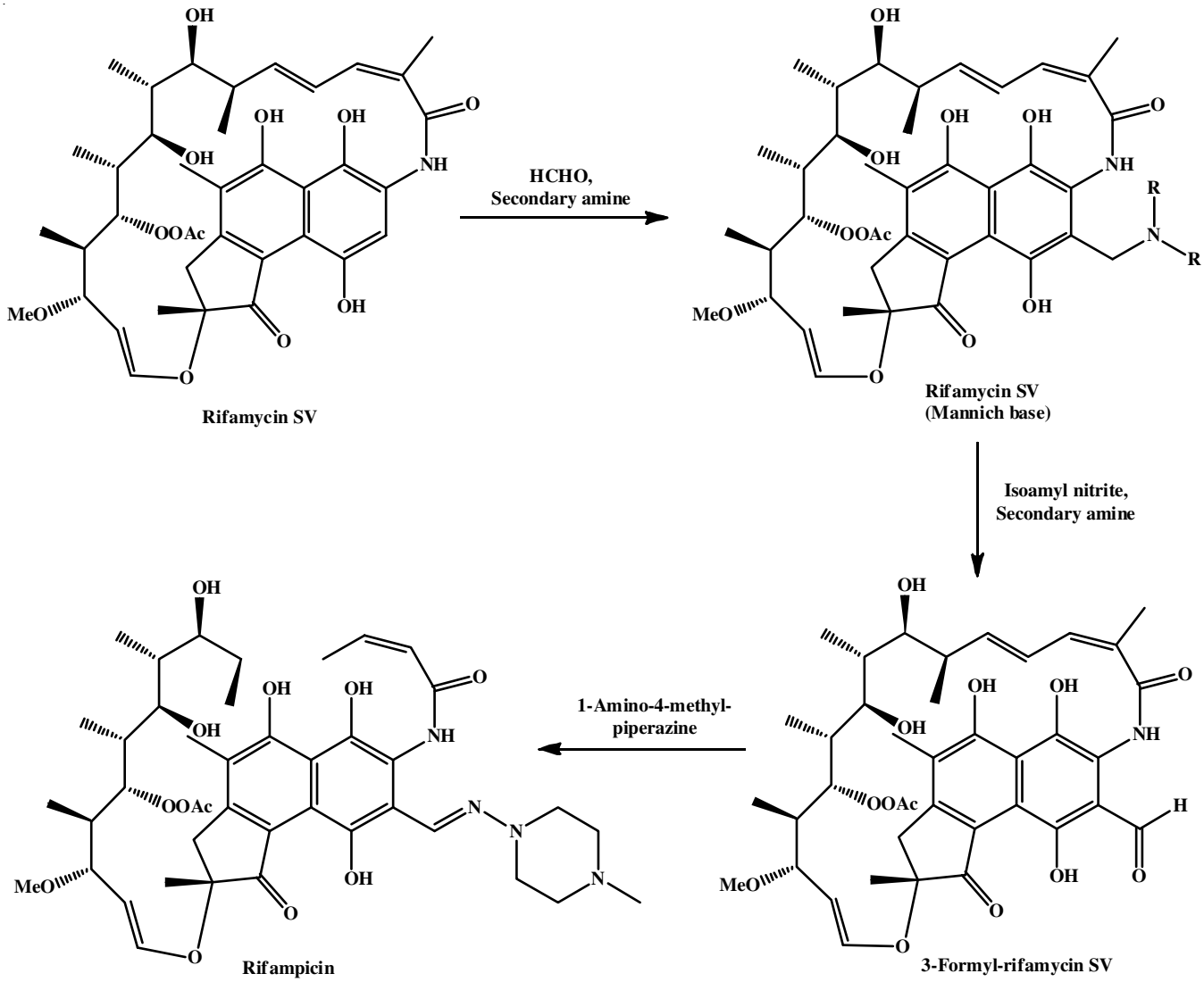

Fig. 10. Reaction with 1-amino-4-methylpiperazine

CPNP contamination in rifapentine can emerge when nitrosating agents (incorporated during the manufacturing stage) react with 1-cyclopentyl piperazine (Fig. 11). The prevalence of CPNP toxicophores (structural and toxicological links) suggests that these nitrosamines are far less oncogenic than NDMA [31].

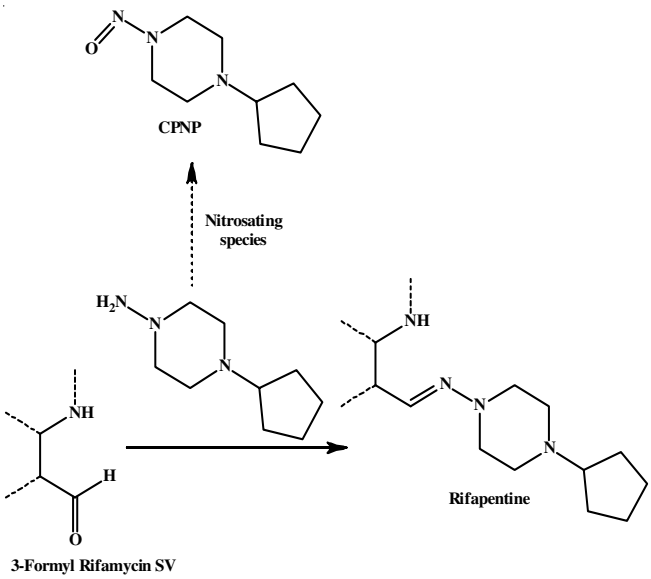

Fig. 11. Possible root cause behind nitrosamine formation during rifapentine synthesis

Streptozotocin (anti-cancer): It is a naturally occurring alkylating antineoplastic agent used in the treatment of some malignancies of the islets of Langerhans. Its structure is similar to that of nitrosamines and the generation of reactive oxygen species induces higher levels of superoxide, nitric oxide and lipid peroxidation, all of which cause DNA damage in streptozotocin-induced cellular injury [37].

Because of their structural similarity to streptozotocin (Fig. 12), de la Monte & Tong [37] investigated the role of nitrosamines in causing cellular damage and disease by functioning as an alkylating agent, mutagen and inducer of DNA adducts, single-strand DNA breakage and the emergence of nitric oxide, superoxide anion, H_2O_2 and OH radicals. NDEA can trigger molecular and metabolic abnormalities in post-mitotic CNS neurons that are similar to the effects of streptozotocin therapy and Alzheimer's disease neurodegeneration [37].

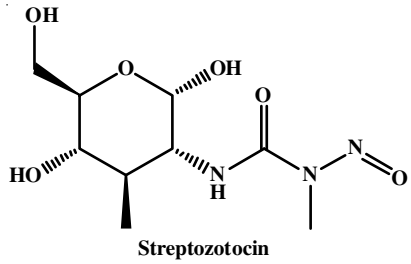

Fig. 12. Structure of streptozotocin

Molsidomine (vasodilators): Molsidomine is a nitrovasodilator that is prescribed for the long-term prevention of angina pectoris, left heart failure and acute myocardial infarction [38]. Since nitric oxide donor has a morpholine containing component, N-nitrosomorpholine was formed (N-Mor). The primary source must be nitrite leftover from manufacturing or a nitrosating substance from tablet excipients. As a result, the interaction with traces of morpholine, a previously described impurity E of molsidomine, is observed (Fig. 13) [38]. The examined molsidomine product was not withdrawn during the quantitative analysis because it was still within its expiration date range. As a result, the formation and evaluation of *N*-nitrosomorpholine should be highlighted.

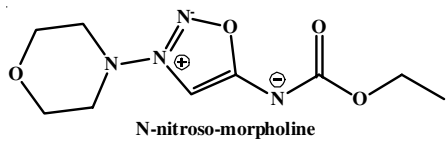

Fig. 13. Structure of the nitric oxide donor molsidomine

Aminophenazone (analgesics): In 1977, the German Bundesgesundheitsamt recommended that aminophenazone (amidopyrine) preparations be withdrawn from the market [39]. Revelation of substantial NDMA residues amongst aminophenazone batches prompted the termination from the market.

Aminophenazone has a non-aromatic pyrazolone ring present at the 4-position with dimethylamine group. Hydrolytic degradation produces 4-hydroxypyrazol-3-one derivative as well as the release of DMA [40,41] (Fig. 14). The formation of NDMA must be anticipated if $NaNO_2$ carryover from the preceding manufacturing step occurs. The withdrawal from the market was associated with the discovery of significant NDMA contamination in API batches (up to 340 ppb). A pre-

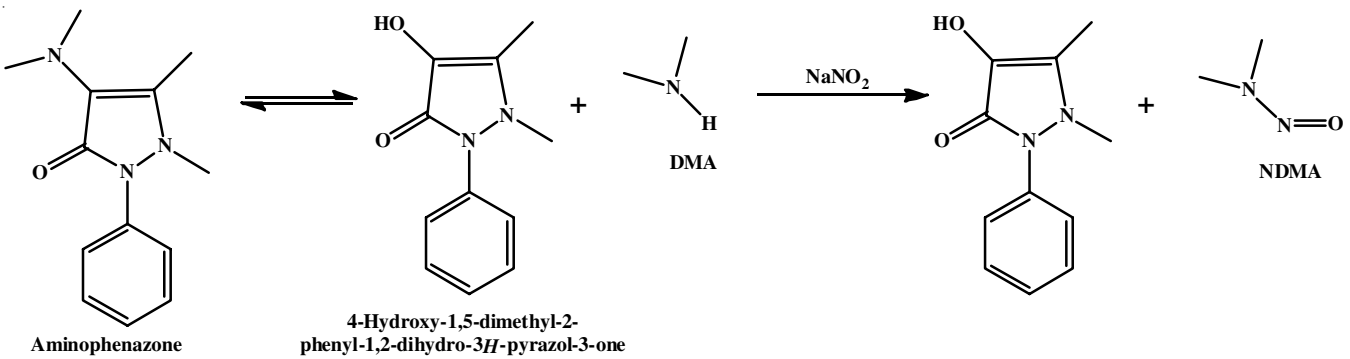

Fig. 14. Generation of NDMA in aminophenazone API

liminary recommendation to incorporate ascorbic (antioxidant) to avert the nitrosation and nitrosamine genesis was over-ruled [5].

Ranitidine (histamine H_2 receptor antagonists): Though NDMA was detected to be present beyond reasonable standards in several ranitidine medicines, however, there are conflicting theories about the source of the impurity, the CHMP evaluated ranitidine medicines and recommended a precautionary suspension of all ranitidine products in the EU [42]. The gradual occurrence of NDMA in ranitidine is being investigated as a possible root cause, but it is unclear whether this impurity can also be derived from ranitidine in gastric fluid once the drug is released from the dosage form and interacts with nitrite-containing food components [8].

A potential reason is the emergence of NDMA during chloramination, which follows a four-step process. In terms of generating NO^+, monochloramine (NH_2Cl) is preferable to dichloramine ($NHCl_2$) (Fig. 15). Because ranitidine is susceptible to degradation, storage conditions are likely to be critical in contributing towards the formation of nitrosamine impurities. Ranitidine degrades rapidly in the presence of heat, humidity, light and oxygen [3,5,36,43].

Nizatidine: Nizatidine is a thiazole derivative of ranitidine, its mechanism of action is similar to other H_2-antagonists, just like its receptor selectivity. Replacement of imidazole ring with a thiazole moiety enhances the potency and selectivity of H_2-antagonism and curbs cytochrome and renal secretory drug interactions [1].

It has been hypothesized that nitrosation of this drug may be attributable to the dimethylamino functional group (Fig. 16). The amount of nitrosamine present in this drug has been found to be less than ranitidine, attributing to the thiazole being less electron rich than the furan substitute in ranitidine. Moreover, since ranitidine exists in hydrochloride salt form, it is more likely to undergo nitrosation as compared to free base form of nizatidine [1].

Other case studies

The following cases are some of the latest drug products wherein nitrosamine impurities are found to be present (Fig. 17):

(i) Fenfluramine is a selective serotonin reuptake inhibitor (SSRI) that elevates serotonin concentrations in synapses in the brain to reduce calorie consumption. Fenfluramine-induced anorexia and weight loss have been documented in a variety of early therapeutic trials. Adachi *et al.* [44] reported a series of cases with liver damage with serious health repercussions (one patient underwent liver transplantation and another died) caused by the consumption of Chinese weight-loss nutraceutical.

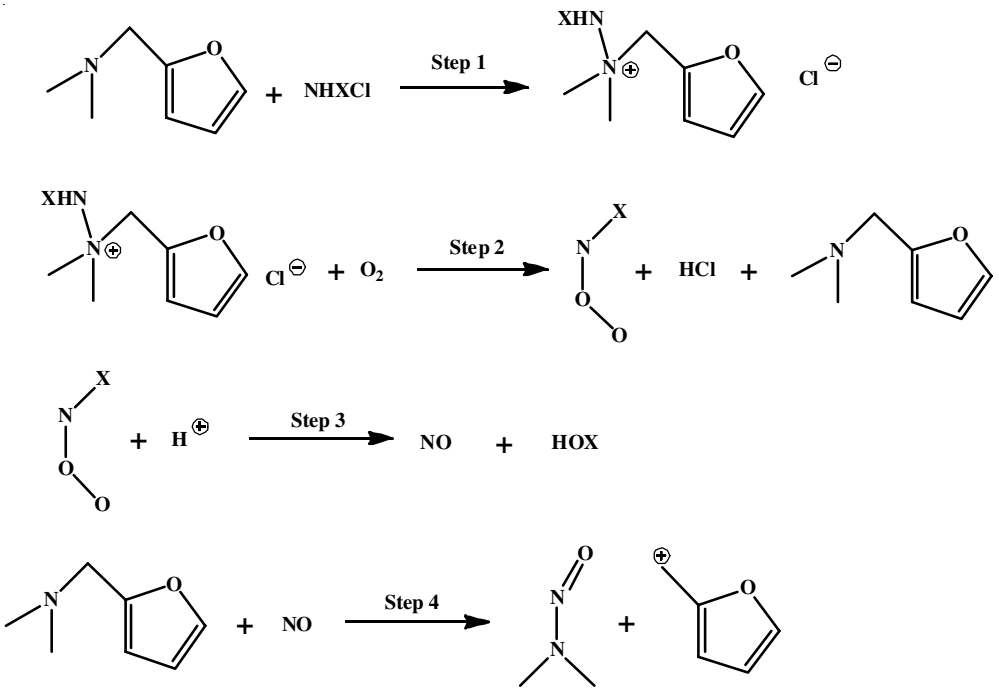

Fig. 15. NDMA formation in ranitidine during chloramination

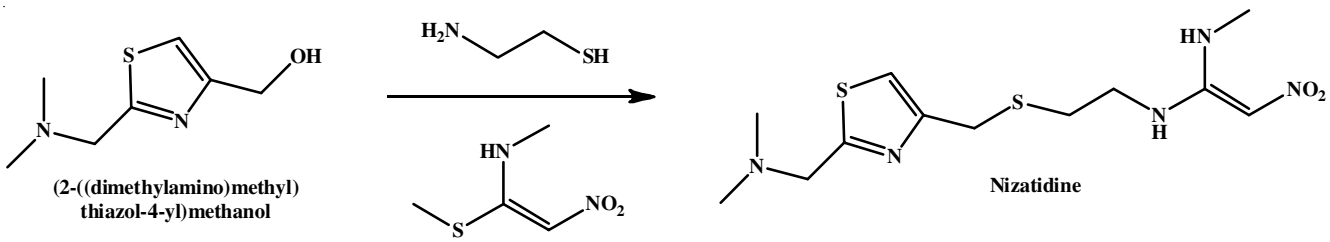

Fig. 16. Synthetic scheme for nizatidine

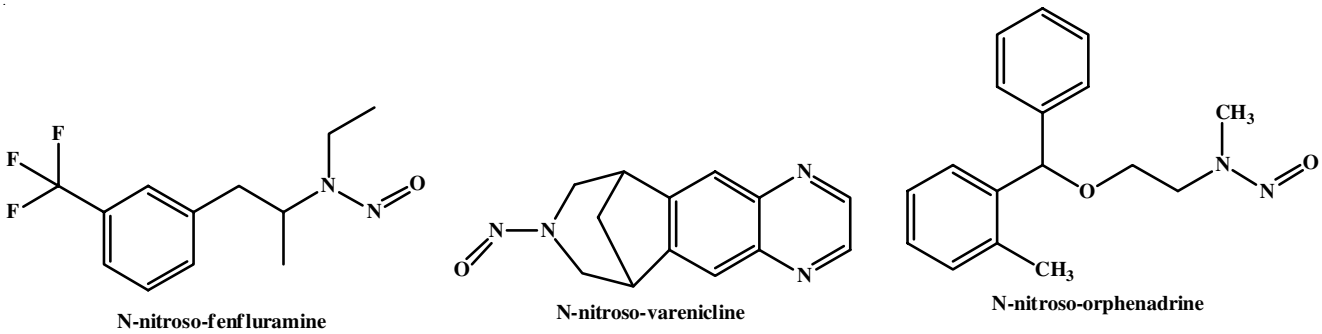

Fig. 17. Nitrosated impurities of the aforementioned drug compounds

N-nitroso-fenfluramine was present in this herbal medicine supplement.

(ii) Varenicline is a prescription medicine used to help in smoking cessation. It is a partial agonist of nicotinic acetylcholine receptor α4/β2 sub-type [45]. The FDA discovered amounts of N-nitroso-varenicline over the FDA's permitted intake limit in several samples of varenicline finished products. All CHAMPIX (varenicline) batches were discovered to contain concentrations of N-nitroso-varenicline above the EU-set permissible level of consumption and were subject to recall.

(iii) Orphenadrine is a muscarinic antagonist which is used to alleviate discomfort associated with acute inflammatory musculoskeletal disorders in conjunction with rest, physical therapy and other interventions. However, the presence of *N*-nitroso-orphenadrine, the most recent recalls [46] have been concentrated on Orphenadrine Citrate ER Tablets.

Role of antioxidants in preventing nitrosamine formation: According to previous research [30,47], high levels of nitrite in free form (mg/kg) have been identified in cosmeceuticals containing 0.01% bronopol as preservative (Fig. 18). The amount of nitrite moiety in such preparations that encompass antioxidants has been substantially lowered, owing to antioxidants' ability to complex free nitrite. Nitrite levels are significantly higher in pharmaceuticals with α-tocopherol (vitamin E) than in those containing butylated hydroxy toluene-an antioxidant, but suppression of nitrosation is similarly efficient. One may concluded that α-tocopherol hinders nitrosamine formation more pertinently by inhibiting the nitrosation reaction *via* free radicals rather than nitrite complexation [47].

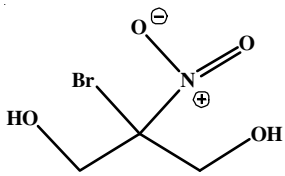

Fig. 18. Chemical structure of bronopol

To be efficacious in both phases, cosmetic preparations constitute both hydrophobic and hydrophilic nitrosation inhibitors [30]. Among the oil-soluble inhibitors include ascorbyl palmitate, ethoxyquin, tocopherols, butylated hydroxytoluene/hydroxyanisole (BHT/BHA). Magnesium ascorbyl phosphate, ascorbic acid, sulfamic acid, sodium citrate and perhaps other antioxidants are sources of water-soluble inhibitors [47].

Carcinogenicity and mutagenicity potential of nitrosamine impurities: N-Nitrosamines pose a risk of being cancer inducing in nature, with a wide range of potency encompassing the most and least potent nitrosamine [48]. Those possessing animal data are designated as IIA/B by IARC. Presently, merely a few tobacco-based nitrosamines are categorized as class I, despite a dearth of adequate substance-specific human data.

N-Nitrosamines must be metabolically activated to form alkydiazonium ions, forerunners of electrophiles that adversely interact with genetic material [15]; their emergence is dependent on the type of N-nitrosamine in question and is associated by metabolic activation by α-hydroxylation.

Different nitrosamines and metabolites may induce an array of DNA lesions. Recently, tobacco-specific *N*-nitrosamines namely *N*-nitroso-nornicotine (produced during tobacco curing and processing) and nicotine-derived nitrosamine ketone (NNK) have gained interest by the scientific community, which are regarded human carcinogens by IARC [49]. *In vivo*, NNK is predominantly activated by cytochrome P450 to intermediates that cause DNA pyridyloxobutylation and methylation as well as single strand breaks [5,33,48,50].

Moreover, nitrate exposure and food intake are substantially ubiquitous than nitrite exposure and intake due to its prevalence and utility in agriculture. Extensive studies have revealed that nitrate undergoes reduction to nitrite in the mouth, which subsequently combines with gastric acid to form nitrous acid and rapidly nitrosate nitrogen molecules. A fraction of nitrate that circulates in the bloodstream is converted to nitrite in saliva. The amount produced by this later pathway is typically greater than that produced by the swift nitrate reduction upon ingestion [17,30].

Conclusion

Since 2018, the structural approach concerning nitrosamine control and awareness in the pharmaceutical realm has shifted radically. To prevent unwarranted risks, marketing authorization holders (MAHs) and regulatory agencies continue to take measures to address concerns about nitrosamines. Analytical agencies worldwide should emphasize upon testing procedures to ascertain positive findings are recognized prior to market release and to eliminate foreign contamination. After the thoughtful consideration of patient safety and statutory practical prerequisites, Committee for Medicinal Products for Human Use (CHMP) affirms on imposing limits for individual nitrosamines in medicines premised on ICH M7 principles

(guidelines on "cohort of concern" substances) and determined based on a lifetime daily exposure is the safest bet. This would assure a steady distribution of pharmaceuticals, along with unequivocal and predictable decision-making. Suitable control strategies and the design or customization of production processes may be critical in mitigating these contaminants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Food and Drug Administration, Control of Nitrosamine Impurities in Human Drugs, Guidance for Industry (Rev. 1), September, p. 24 (2021); https://www.fda.gov/media/141720/download
2. International Agency for Research on Cancer, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, vol. 1-42, IARC; Lyon, France (1987).
3. J.C. Beard and T.M. Swager, *J. Org. Chem.*, **86**, 2037 (2021); https://doi.org/10.1021/acs.joc.0c02774
4. R. Boetzel, J. Schlingemann, S. Hickert, C. Korn, G. Kocks, B. Luck, G. Blom, M. Harrison, M. François, L. Allain, Y. Wu and Y. Bousraf, *J. Pharm. Sci*., (2022); https://doi.org/10.1016/j.xphs.2022.04.016
5. European Medicines Agency, Assessment Report: Nitrosamine Impurities in Human Medicinal Products, EMA/369136/2020, vol. 5, no. June, pp. 1-90 (2020); https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf
6. Y. Wu, J. Levons, A.S. Narang, K. Raghavan and V.M. Rao, *AAPS PharmSciTech*, **12**, 1248 (2011); https://doi.org/10.1208/s12249-011-9677-z
7. G.W. Sluggett, T. Zelesky, E.M. Hetrick, Y. Babayan and S.W. Baertschi, *J. Pharm. Biomed. Anal*., **149**, 206 (2018); https://doi.org/10.1016/j.jpba.2017.10.035
8. M.J. Giridharan and R. Srinivasan, *Ann. Roman. Soc. Cell Biol.*, **25**, 1633 (2021).
9. M.K. Parr and J.F. Joseph, *J. Pharm. Biomed. Anal.*, **164**, 536 (2019); https://doi.org/10.1016/j.jpba.2018.11.010
10. R. Zou, X. Liao, L. Zhao and B. Yuan, *Environ. Sci. Pollut. Res.*, **25**, 13489 (2018); https://doi.org/10.1007/s11356-018-1470-z
11. J.L. Roux, J. Croue, M. Deborde, S. Papot and M. Deborde, *Environ. Sci. Technol.*, **46**, 11095 (2012); https://doi.org/10.1021/es3023094
12. M. Hazarika, Nitrosamine-A Big No for Printing Inks for Food and Pharma Packaging, The Packman Mag., February 16 (2020); https://thepackman.in/nitrosamine-a-big-no-for-printing-inks-for-food-and-pharma-packaging/
13. European Medicines Agency, Information on Nitrosamines for Marketing Authorisation Holders, Ema/189634/2019, vol. 5, pp. 1-5 (2019); https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-information-nitrosamines-marketing-authorisation-holders-obsolete_.pdf
14. J.P. Dattatraya, S.N. Arun, V.K. Deshmukh, V. B. Ghawate and A.R. Pund, *Pharm. Resonance*, **4**, 44 (2022).
15. C. Adoption and C. Date, ICH Guideline M7 (R1) on Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, vol. 7, no. 2 (2018).
16. K. Li, K. Ricker, F.C. Tsai, C.Y.J. Hsieh, G. Osborne, M. Sun, M.E. Marder, S. Elmore, R. Schmitz and M.S. Sandy, *Int. J. Environ. Res. Public Health*, **18**, 9465 (2021); https://doi.org/10.3390/ijerph18189465
17. J. Schlingemann, M.J. Burns, D.J. Ponting, C.M. Avila, N.E. Romero, M.A. Jaywant, G.F. Smith, I.W. Ashworth, S. Simon, C. Saal and A. Wilk, *J. Pharm. Sci*., (2022); https://doi.org/10.1016/j.xphs.2022.11.013.2022
18. European Medicines Regulatory Network Approach for the Implementation of the CHMP Opinion Pursuant to Article 5(3) of Regulation (EC) No. 726/2004 for Nitrosamine Impurities in Human Medicines, vol. 5, no. 726 (2021).
19. J.M. Kemper, S.S. Walse and W.A. Mitch, *Environ. Sci. Technol.*, **44**, 1224 (2010); https://doi.org/10.1021/es902840h
20. D.J. Snodin, *Org. Process Res. Dev.*, **24**, 2407 (2020); https://doi.org/10.1021/acs.oprd.0c00343
21. R. Thomas, R.E. Tennant, A.A.F. Oliveira and D.J. Ponting, *Chem. Res. Toxicol.*, **35**, 1997 (2022); https://doi.org/10.1021/acs.chemrestox.2c00199
22. R. Thomas, R. Ponting and R. Thresher, *Regul. Toxicol. Pharmacol.*, **121**, 104875 (2021); https://doi.org/10.1016/j.yrtph.2021.104875
23. USFDA, No Title; https://www.fda.gov/drugs/news-events-human-drugs/nitrosamines-impurities-drugs-health-risk-assessment-and-mitigation-public-workshop-03292021
24. R.P. Hanzlik and K.H.J. Ling, *J. Org. Chem.*, **55**, 3992 (1990); https://doi.org/10.1021/jo00300a010
25. D.A. Smith, L. Di and E.H. Kerns, *Nat. Rev. Drug Discov.*, **9**, 929 (2010); https://doi.org/10.1038/nrd3287
26. Z. Zhivkova and I. Doytchinova, *J. Pharm. Sci.*, **101**, 4627 (2012); https://doi.org/10.1002/jps.23303
27. P. Gardiner, R.J. Cox and K. Grime, *Drug Metab. Dispos.*, **47**, 865 (2019); https://doi.org/10.1124/dmd.119.087163
28. R. Lonsdale, K.T. Houghton, J. Zurek, C.M. Bathelt, N. Foloppe, M.J. De Groot, J.N. Harvey and A.J. Mulholland, *J. Am. Chem. Soc.*, **135**, 8001 (2013); https://doi.org/10.1021/ja402016p
29. D.F. Lewis and Y. Ito, *Expert Opin. Drug Metab. Toxicol.*, **6**, 661 (2010); https://doi.org/10.1517/17425251003674380
30. N.R. Loeppky, Nitrosamine and N-Nitroso Compound Chemistry and Biochemistry, Advances and Perspectives; ACS Symposium Series, vol. 553, Chap. 1, pp. 1-8 (1994).
31. B. Jose, E.J. Jesy and R.J. Nedumpara, *World J. Pharm. Res.*, **3**, 5041 (2014); https://doi.org/10.20959/wjpr202111-21490
32. P.V. Ajith Kumar and M. Deepa, *Int. J. Res. Med. Sci.*, **8**, 3778 (2020); https://doi.org/10.18203/2320-6012.ijrms20204273
33. B. Tuesuwan and V. Vongsutilers, *J. Pharm. Sci.*, **110**, 3118 (2021); https://doi.org/10.1016/j.xphs.2021.04.021
34. D.A. Keire, R. Bream, U. Wollein, J. Schmaler-Ripcke, A. Burchardt, M. Conti, A. Zmyslowski, P. Keizers, J. Morin, J. Poh, M. George and M. Wierer, *AAPS J.*, **24**, 56 (2022); https://doi.org/10.1208/s12248-022-00702-4
35. S.C. Jagdale, S.A. Patil, B.S. Kuchekar and A.R. Chabukswar, *J. Young Pharm.*, **3**, 197 (2011); https://doi.org/10.4103/0975-1483.83758
36. H. Yokoo, E. Yamamoto, S. Masada, N. Uchiyama, T. Hakamatsuka, G. Tsuji, Y. Demizu, K. Izutsu and Y. Goda, *Chem. Pharm. Bull. (Tokyo)*, **69**, 872 (2021); https://doi.org/10.1248/cpb.c21-00289
37. S.M. De La Monte and M. Tong, *J. Alzheimers Dis*., **17**, 817 (2009); https://doi.org/10.3233/JAD-2009-1098
38. S. Schmidtsdorff, J. Neumann, A.H. Schmidt and M.K. Parr, *Arch. Pharm*., **356**, 2200484 (2023); https://doi.org/10.1002/ardp.202200484
39. G. Eisenbrand, B. Spiegelhalder, J. Kann, R. Klein and R. Preussmann, *Arzneimittelforschung*, **29**, 867 (1979); https://doi.org/10.1002/chin.197938327
40. J. Reisch, H. Alfes, N. Jantos and H. Möllmann, *Acta Pharm Suec*., **5**, 393 (1968).
41. J. Reisch, R. Pagnucco, H. Alfes, N. Jantos and H. Möllmann, *J. Pharm. Pharmacol*., **20**, 81 (1968); https://doi.org/10.1111/j.2042-7158.1968.tb09693.x
42. I. Sedlo, T. Kolonic and S. Tomic, *Arh. Hig. Rada Toksikol*., **72**, 1 (2021); https://doi.org/10.2478/aiht-2021-72-3491

43. Y.D. Liu, M. Selbes, C. Zeng, R. Zhong and T. Karanfil, *Environ. Sci. Technol.*, **48**, 8653 (2014); https://doi.org/10.1021/es500997e
44. M. Adachi, H. Saito, H. Kobayashi, Y. Horie, S. Kato, M. Yoshioka and H. Ishii, *Ann. Intern. Med.*, **139**, 488 (2003); https://doi.org/10.7326/0003-4819-139-6-200309160-00012
45. D. Gonzales, S.I. Rennard, M. Nides, C. Oncken, S. Azoulay, C.B Billing, E.J. Watsky, J. Gong, K.E Williams and K.R Reeves, *JAMA*, **296**, 47 (2006); https://doi.org/10.1001/jama.296.1.47
46. Sandoz, Inc. Issues Nationwide Recall of 13 Lots of Orphenadrine Citrate 100mg Extended Release Tablets Due to Presence of a Nitrosamine Impurity, March 22 (2022); https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/sandoz-inc-issues-nationwide; Accessed on 5th March 2022.
47. P.C. Dunnett and G.M. Telling, *Int. J. Cosmet. Sci.*, **6**, 241 (1984); https://doi.org/10.1111/j.1467-2494.1984.tb00381.x
48. A.B. Patel, A.H. Asnani, A.J. Vyas, N.K. Patel, A.I. Patel and A.N. Lumbhani, *Asian J. Pharm. Res.*, **10**, 187 (2021); https://doi.org/10.52711/2231-5691.2021.00034
49. E.S. Carlson, P. Upadhyaya and S.S. Hecht, *Chem. Res. Toxicol.*, **29**, 2194 (2016); https://doi.org/10.1021/acs.chemrestox.6b00384
50. S.Y. Lee and J.B. Guttenpbn, *Carcinogenesis*, **2**, 1339 (1981); https://doi.org/10.1093/carcin/2.12.1339