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Nitrosamine Contamination in Pharmaceuticals: A Comprehensive Review on Nitrosation Pathways, Potential Root Cause, Detection, Risk Assessment, and Mitigation Strategies

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N-nitrosamines, recognized as potentially fatal and likely human carcinogens, have been detected in various medications, including antidiabetic drugs and Histamine-2 receptor blockers, particularly those with specific amine structures. This contamination has prompted regulatory bodies to implement rigorous testing and mitigation strategies to safeguard public health. This review offers a concise overview of nitrosamine impurities, their precursors, affected medications, and associated health risks. Current regulations stress the need for robust analytical procedures to detect and mitigate nitrosamines, such as using nitrite scavengers in oral medications. Despite these efforts, challenges remain in accurately assessing risks and integrating effective detection methods. Key findings indicate a need for continuous monitoring, advancements in detection technologies, and the development of comprehensive risk assessment frameworks. Recommendations include adopting proactive risk management strategies, enhancing industry collaboration for better data sharing, updating regulatory guidelines, and incorporating country-specific risk mitigation efforts to address emerging threats effectively.

Keywords: Analytical Method; Carcinogenic nitrosamine impurities; Nitrosamine formation; Nitrite scavenger; Risk Evaluation.

Since July 2018, global regulatory agencies have flagged angiotensin II receptor blockers (ARBs)due to suspected contamination with nitrosamines, including the carcinogen N-nitroso dimethylamine (NDMA) found in valsartan[1]. As a result, the Food and drug administration (FDA) in the United states (US) issued voluntary recalls, and recalls were implemented widely throughout Europe. Next, it was discovered that losartan and valsartan contained N-nitrosoN-methyl-4-aminobutyric acid (NMBA) and N-nitrosodiethylamine (NDEA), respectively. Efforts are underway to investigate nitrosamine contamination in ARB formulations, with concerns centered around tetrazole formation during Active Pharmaceutical Ingredient (API) production. The contamination issue has extended to other drugs like ranitidine and metformin, where NDMA contamination led to market withdrawal of ranitidine in 2020 [2,3]. Ranitidine's NDMA

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levels can increase over time, especially at higher temperatures. Metformin's NDMA contamination is likely due to finished product degradation under specific conditions [4].

Brief Evolution of the Generation of N-Nitrosamines in Pharmaceutical Products

Concerns about N-nitrosamines (NAs) began in the late 1800s, but their dangers became evident in 1954, leading to their classification as carcinogens in 1970. They are now found in tobacco, food, medicine, and possibly the gut [5] . International council for harmonization (ICH) guideline M7R1confirmed this classification, classifying N-nitrosamines and their precursors as members of a "cohort of concern" because of their high mutagenic potency. Significant drug recalls involving N-nitrosamine have occurred in recent years [6]. The recalls included valsartan, angiotensin II receptor antagonists, anti-diabetic medications, and ranitidine due to harmful impurities[7]. Regulations to prevent such contamination were incorporated in replies from regulatory agencies like the European medicine agency (EMA) and FDA[8,9]. Additionally, research on the use of nitrite scavenger's dates back to the 1960s, when it was discovered that ascorbic acid might decrease the formation of N-nitrosamines by converting nitrous acid more precisely, the anhydride Dinitrogen trioxide (N_2O_3) to Nitric oxide (NO) [10]. Studies carried out in the 1970s focused on inhibiting agents, namely the interaction between ascorbic acid and nitrite, in order to reduce nitrosation [11]. This preventive measure was tested in various pharmaceutical systems, including piperazine and tetracycline drugs, to reduce nitrosamine synthesis[12].

Potential origins of nitrosamine impurities in pharmaceuticals

Angiotensin II receptor blockers (ARBs)

Nitrosamine impurities in ARBs, particularly tetrazole-based ones like losartan and valsartan, result from nitrosation during API production, often linked to recycled catalysts or solvents. Non-tetrazole ARBs, like telmisartan and eprosartan, are less affected [13]. To avoid using hazardous hydrazoic acid, azides and nitriles can be converted into a tetrazole ring through a [1 + 3] cycloaddition process[14]. The formation of tetrazole using azide reagents is typically carried out in the concluding stage of sartan synthesis [15] . Despite their human health hazards, organometallic azide derivatives like trimethyltin azide (Me_3SnN_3) and tributyltin azide ($BuSnN_3$) and sodium azide (NaN_3) are preferred as azide reagents because they are easy to handle and dispose of. Sodium nitrite is usually added in an acidic environment to neutralize any unreacted azides that remain after the tetrazole production process[16]. The disposal of alkyl amine residues, unreacted azides, or contaminated solvents can unintentionally form nitrosamines, posing a contamination risk. The recall of valsartan, the first ARB with NDMA presence, was linked to NDMA forming from dimethylamine (DMA), a residual impurity in N,N-dimethylformamide (DMF) used in API synthesis[17]. During ARB production, DMF can degrade into dimethylamine (DMA), while triethylamine (TEA) used in losartan synthesis may be contaminated with diethylamine (DEA), which is linked to the presence of NDEA [18] which is a known precursor of NDEA as reported previously. Additionally, TEA can undergo direct dealkylation to form DEA when exposed to nitrous acid or nitrite, as shown in Figure 1. Additional nitrosamine compounds include N, Nitrosodiisopropylamine (NDIPA), NMBA and N- nitrosoethylisopropylamine (NEIPA) have also been detected in ARBs in addition to NDEA. Initially, NMBA contamination was detected in both irbesartan and losartan. The precursor of NMBA is N-Methyl amino N-butyric acid (MBA), that might develop as a result of N-methylpyrrolidinone (NMP) deterioration, an organic solvent utilized during tetrazole synthesis. N-ethyl N-isopropylamine (EIPA) and N,N -diisopropylamine (DIPA), which act as the corresponding precursors of NEIPA and NDIPA, are examples of dealkylative compounds that can be produced from NDEA, N,N -diisopropylethylamine (DIPEA), a frequent base in API synthesis. Tetrazole-containing ARBs may be contaminated with various nitrosamines due to different amine reagents used in their synthesis. Risk assessments and manufacturer recalls have identified azido impurities as novel by-products, although the nitrosamine issue has not been fully resolved [19]. Azido impurities, specifically 4- (azidomethyl)-[1,10-biphenyl]-2-carbonitrile and 5-(4'-(azidomethyl)-[1,10-biphenyl]-2-yl)-1H-tetrazole (AZTT), arise from a secondary reaction between sodium azide and the left-over intermediates from the step before it. ARB

medications with azido impurities, like losartan forming AZTT, have led to recalls by the Canadian FDA. To reduce nitrosamine contamination, avoiding nitrosating agents is advised. China uses a risky hydrogen peroxide quenching method for losartan, while triaryl phosphine can alternatively remove residual azides via the Staudinger reaction [20]. In a European patent, the utilization of triphenylphosphine for azide quenching has been described as a method for in-process remediation [21]. Triphenylphosphine reacts with an azide to form a phosphazide intermediate, which releases nitrogen (N_2) to generate iminophorane. An aqueous workup then yields amine derivatives and phosphine oxide. To prevent nitrosamine contamination during ARBs synthesis, use minimal triphenylphosphine instead of sodium nitrite for azide removal.

Histamine -2 (H2) receptor antagonists

NDMA, associated with the ozonation and chlorination processes, raises environmental issues [22]. Efforts to remove NDMA revealed a link between ranitidine and NDMA during wastewater treatment. The dimethylamine side chain and nitro group in nizatidine and ranitidine are associated with increased NDMA formation [23]. Ranitidine can produce NDMA directly, while its impurities release NDMA at a notably faster rate. The proposed mechanism involves impurity degradation, inherent instability of the API, and contamination during the manufacturing process [24] . Shown in figure 2.

Ranitidine's NDMA contamination increases with storage time and temperature, with a strong link between ranitidine, DMA, and NDMA formation, raising potential cancer risks [25]. Controlling and keeping an eye on stability-related parameters including temperature, light, and humidity during drug production and storage is advised as a solution to this problem [26]. Ranitidine's temperature-dependent NDMA production requires controlled storage: 2-8°C for parenteral forms and below 25°C for solid forms. Regular NDMA level checks during storage are advised, and a comprehensive strategy to assess and mitigate NDMA risks in ranitidine is essential for safety.

Anti-diabetic drugs

Metformin, a guanidine derivative like dimethyl guanidine, lacks a nitro group unlike ranitidine. Thus, nitrosamine synthesis requires an external nitro-sating agent [27].

Health officials are concerned about NDMA contamination in metformin, linked to environmental factors and insufficient breakdown mechanisms (Figure 3). A 2019 study identified degradation by-products, and the FDA found excess NDMA in 2020 [28] Top of FormBottom of Form. Nitrosation during wet granulation and drying can cause NDMA contamination in tablets. Avoiding nitrate-containing excipients and implementing strict quality control for metformin and pioglitazone is essential to protect patient safety.

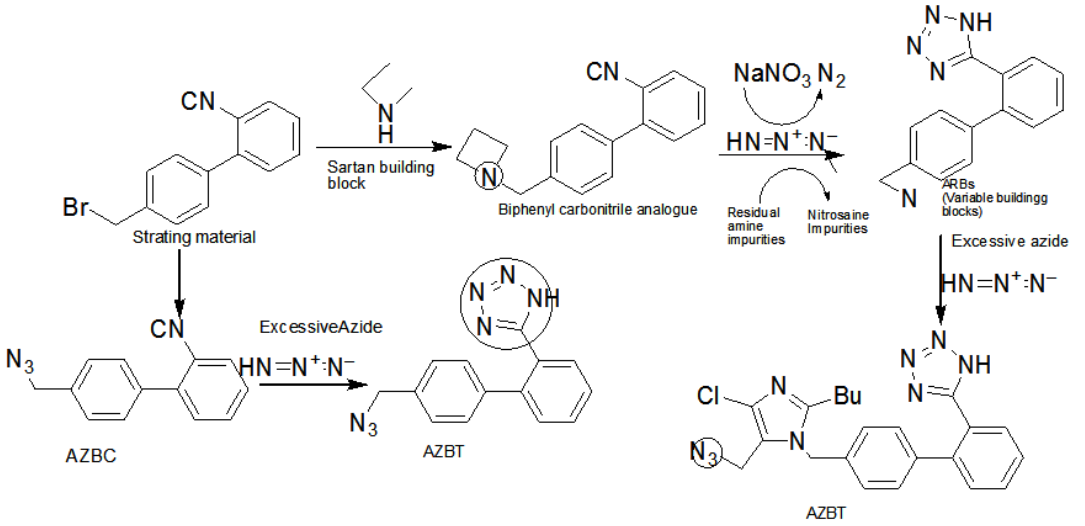

Fig. 1. Possible root causes of nitrosamines and azido impurities in angiotensin II receptor blockers

Antimicrobial agents

In August 2020, the FDA detected nitrosamine impurities 1-cyclopentyl-4-nitrosopiperazine (CPNP) and 1-methyl-4-nitrosopiperazine (MNP) in rifapentine and rifampin (Figure 4), used for tuberculosis. CPNP likely forms during rifapentine synthesis, while MNP's source remains unclear, possibly linked to rifampicin. Many US products exceed acceptable nitrosamine limits, with CPNP in rifapentine at 0.1 ppm and MNP in rifampin at 0.16 ppm. The FDA set higher limits (20 ppm for CPNP and 5 ppm for MNP) to ensure drug availability, while Singapore's Health Sciences Authority (HAS) approved rifampin with trace MNP.

Nitrosamine toxophores generally imply lower carcinogenicity than NDMA[29]. NDMA's interim Acceptable Intake (AI) replaces strict limits, requiring manufacturers to prevent contamination and regulators to enforce oversight. Rifapentine and Rifampicin may be contaminated with CPNP and MNP via nitrosating chemicals reacting with piperazine[30]. This emphasizes Strict quality control is vital for detecting and removing contaminants that endanger patient safety during pharmaceutical production.

Other Medicine

Nitrosamine detection in ARBs has highlighted tetrazole-containing drugs, like sartans, as prone to contamination, affecting medications like ceftezole and letrozole, leading to shortages, treatment changes, and wastewater concerns [31].Chloramine disinfection can lead to nitrosamine formation,[32] in APIs like nizatidine, ranitidine, clarithromycin, metformin, and others. Lidocaine may be contaminated with NDEA. Ozonation in sewage treatment helps minimize NDMA formation by facilitating N-oxide production through electron donation [33]. Nitrosamine formation in wastewater, linked to amine-based medicines, challenges drug manufacturers to ensure medication safety through rigorous risk assessment and quality control.

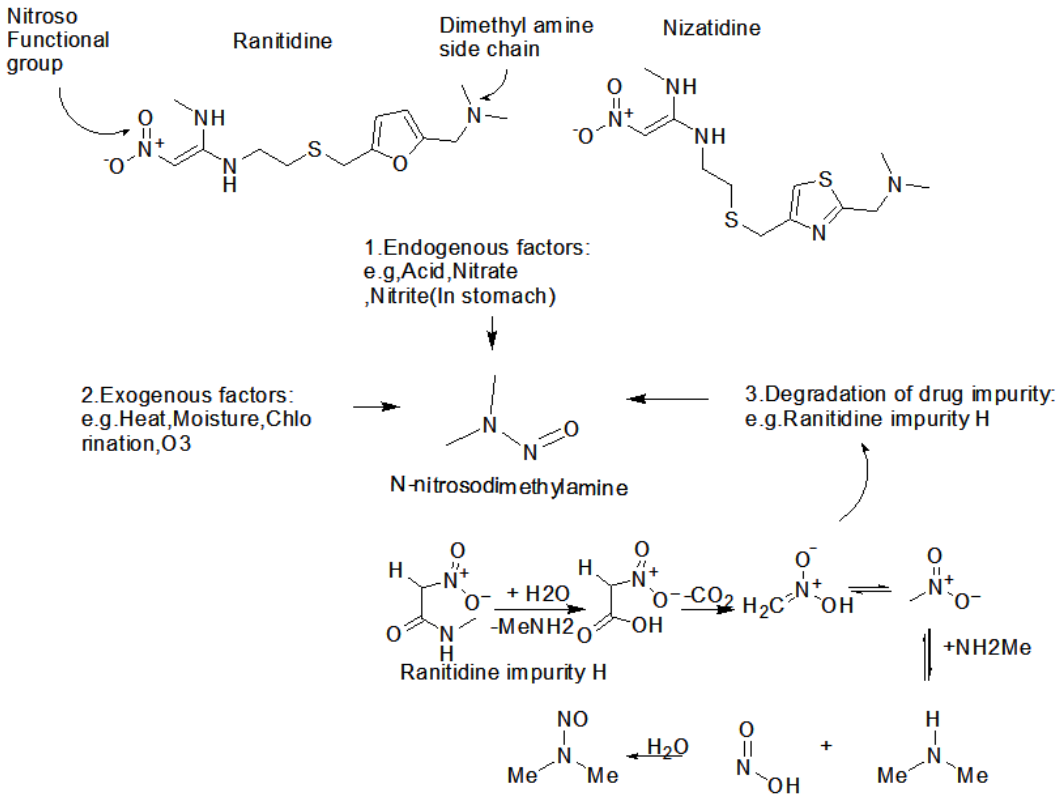

Fig. 2. Possible root causes of N-nitroso dimethylamine (NDMA) contamination in ranitidine and nizatidine and their pharmaceutical products

Amines as N-Nitrosamine Precursors

Nitrosamines in pharmaceuticals are a critical concern due to their carcinogenic potential. They can originate from APIs, solvents, reagents, and degradation during storage. Nitrosamines form via nitrosation of amines by nitrosating agents, with formation influenced by pH, strong acids reduce reactivity and nitrosamine levels, while very low pH decreases nitrosation. Common nitro-sating agents include Nitrous acid (HNO_2), nitrite (NO_2), N_2O_3, Dinitrogen tetroxide (N_2O_4), Nitrosyl Chloride (NOCl), nitrosonium tetrafluoroborate ($NOBF_4$), and nitro thiocyanate (CN_2OS). Controlling these conditions can significantly reduce nitrosamine formation, ensuring product safety and regulatory compliance[34].

Nitrosation Mechanisms for Different Amines (Figure 5)

Primary Amines

The compound is susceptible to undergoing nitrosation, a chemical process where it reacts to form unstable N-nitroso compounds. These

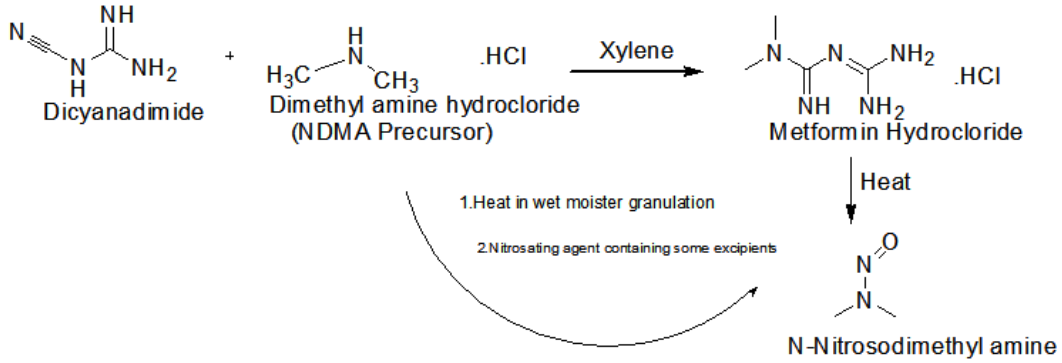

Fig. 3. Possible root causes of N-nitroso dimethylamine (NDMA) contamination in metformin and its pharmaceutical products

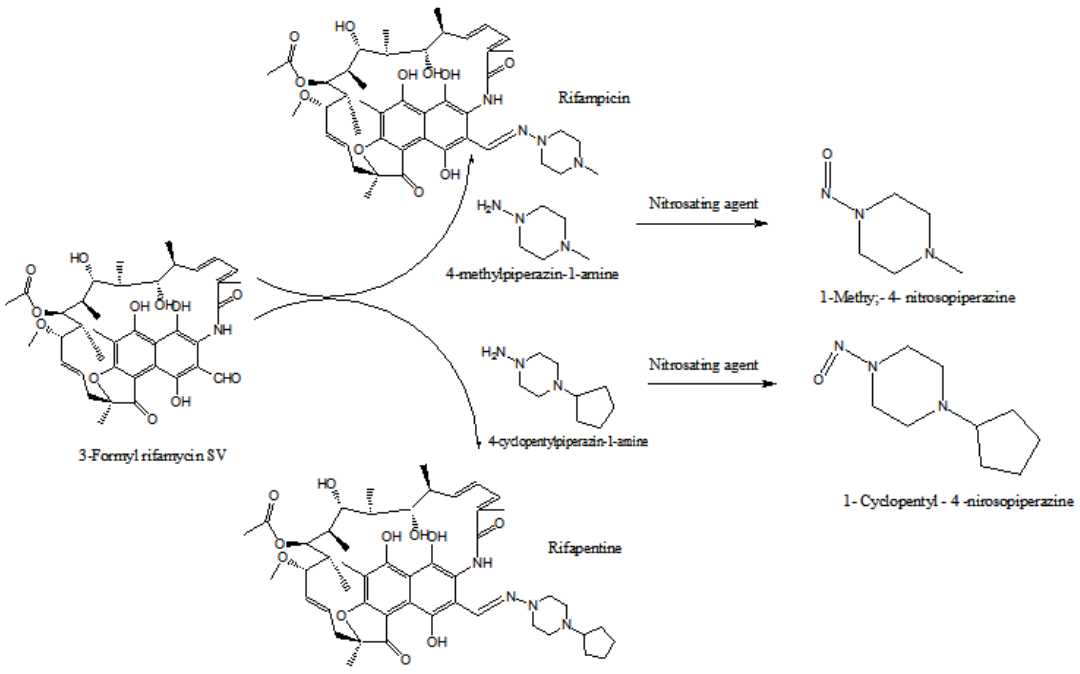

Fig. 4. Potential root causes of contamination with MNP in rifampin and CPNP in rifapentine

compounds can subsequently decompose into diazonium salts which are reactive intermediates in various chemical reactions.

Secondary Amines

Convert to nitrosamines more promptly, making them the most reactive in forming nitrosamine contaminants.

Tertiary amines

This compound can react with nitrites to form N-nitrosamines, following dealkylation to produce secondary amines [35].

Geno toxicity and carcinogenicity in nitrosamines

Nitrosamine contaminants, such as NDEA, present genotoxic risks in pharmaceuticals and are bioactivated by CYP2A6 and cytochrome P450 enzymes [36]. CYP2E1 converts nitrosamines into á-hydroxy nitrosamine, forming alkylating electrophiles that bind Deoxyribonucleic acid (DNA) and proteins, creating adducts[37,38]. Alkylating agents target DNA's N^7 guanine, causing SN^2 alkylation. Alkyl diazonium ions can also target O^4 of thymine and O^6 of guanine, leading to stable DNA modifications that may result in mutations and potentially cause cancer. For example, Translation changes from G: A can occur when O^6 methylguanine and O^6 ethylguanine adducts are misinterpreted as adenine during DNA replication. This misinterpretation can lead to point mutations, altering the genetic code. O^4-alkylthymine adducts are mistakenly recognized as cytosine during DNA replication, which result in translation alterations from T: A to C: G. In vivo experiments on rat and mouse livers exposed to NDEA or NDMA have shown the separation of Ethyl and Methyl DNA adducts, mainly at N^7G, however alkylation at other sites such as O^2T, O^6G, N^3A and O^4T was also

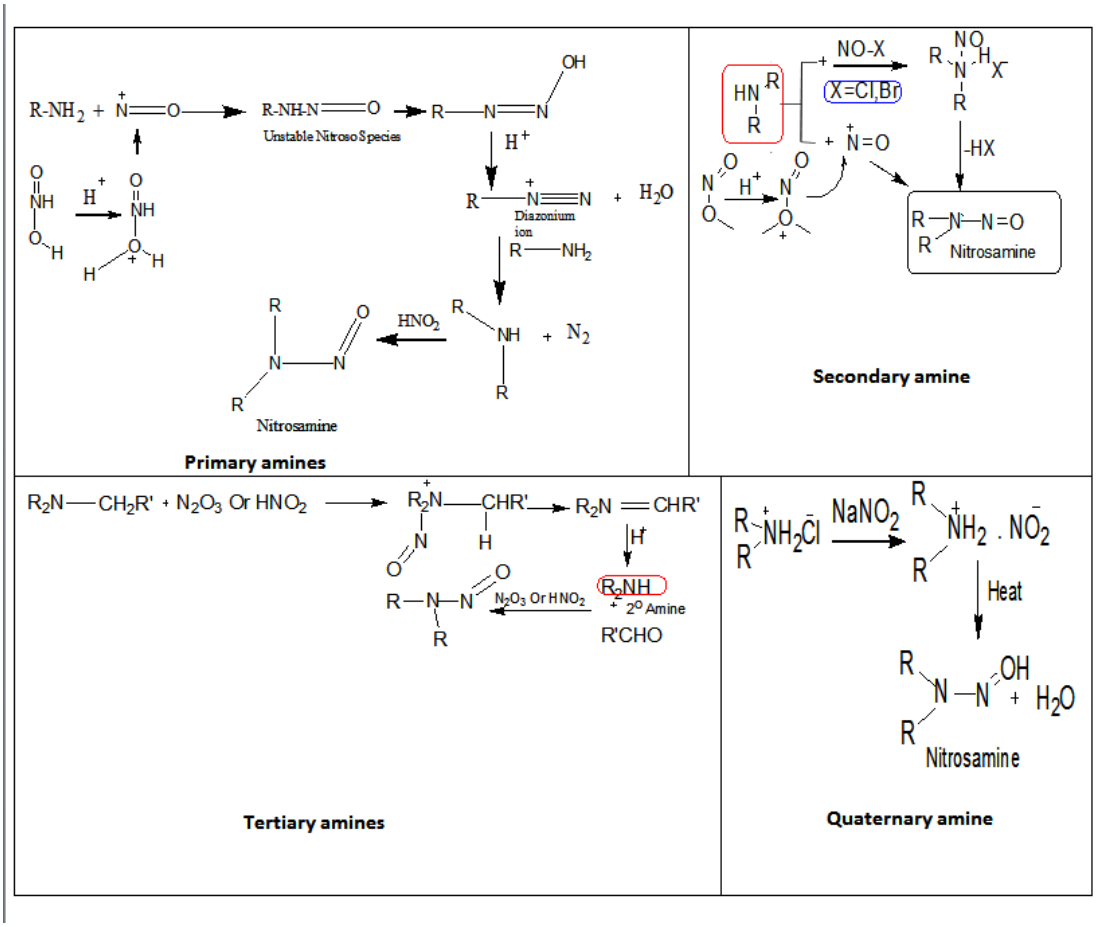

Fig. 5. Pathways of nitrosamine formation between nitrosating agents and primary, secondary, tertiary amines and quaternary amines.

noted, [39] Compared to NDMA, NDEA generated a greater ratio of O^6G to N^7G adducts, which suggests that different alkylation sites on guanines have different distributions shown in Figure 6. Aldehydes produced during the bioactivation of N-nitroso dialkylamines, such as formaldehyde a carcinogen classified by World health organization/ International agency For Research on Cancer (WHO/IARC), form DNA adducts and contribute to the genotoxic effects of alkyl diazonium ions [40]. NDMA and other N-nitroso dialkylamines are strong carcinogens as they alkylate DNA via aldehydes and alkyl-diazonium ions, resulting in increased DNA damage. This is demonstrated by the detection of DNA adducts such as N6-hydroxymethyl-2'-deoxyadenosine and di-(N6-deoxyadenosyl)-methane in the lungs and liver of rats following NDMA injections, which can cause mutations and cancer [41,42] .NDMA and NDEA generate reactive oxygen species (ROS) in Caco-2 cells, leading to disrupted gene expression [43]. Since 1978, the IARC has confirmed that animals exposed to NDMA and NDEA are suitable models for studying their combined carcinogenic effects [44].

According to the ICH M7R1 guideline, consuming N-nitrosamines at interim-limit levels over a lifetime would result in fewer than one additional cancer case per 100,000 people[46]. The EMA flagged potential cancer risks from prolonged NDMA exposure in valsartan, but a Danish study found no significant risk, with an adjusted hazard ratio of 1.09 (95% Confidence interval: 0.85–1.41). These findings highlight the need for strict regulatory oversight to ensure drug safety.

Compounds capable of inhibiting or preventing the development of N-nitrosamines of in pharmaceutical product

Substances can reduce N-nitrosamine synthesis via three main mechanisms: nitration of phenol, redox pathways, and diazotization of primary amines as shown in figure 7 [47]. Effective blocking agents convert nitro-sating agents into non-nitrosating NO, competing with amines based on their quantities [48]. Lipid and

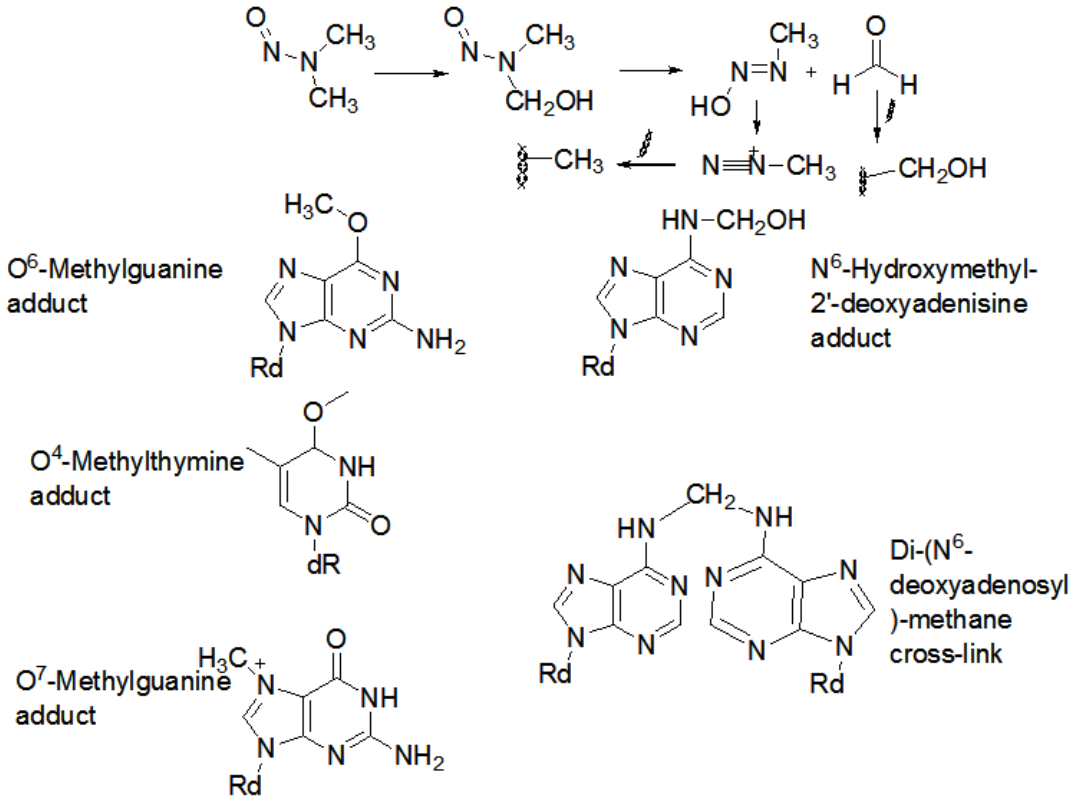

Fig. 6. Bioactivation of nitrosamines to reactive species and the major nucleobase adducts subsequently formed

water-soluble nitrite scavengers, like ascorbic acid, bisulfite, and cysteine, block nitrosation, though phenolics may facilitate trans-nitrosation [49]. Fat-soluble antioxidants like á-tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are also used, but their efficacy may be limited. Solid oral dosage forms can benefit from FDA approved "inactive ingredients" [50]. Table 1 lists nitrite scavengers suitable for these forms.

Ascorbic Acid

Ascorbic acid effectively counteracts various nitro-sating agents and is safe for pharmaceutical use. It excels in weakly acidic and aqueous environments, producing NO from N_2O_3, Nitryl ion (H_2NO,), and NOX [48]. Despite

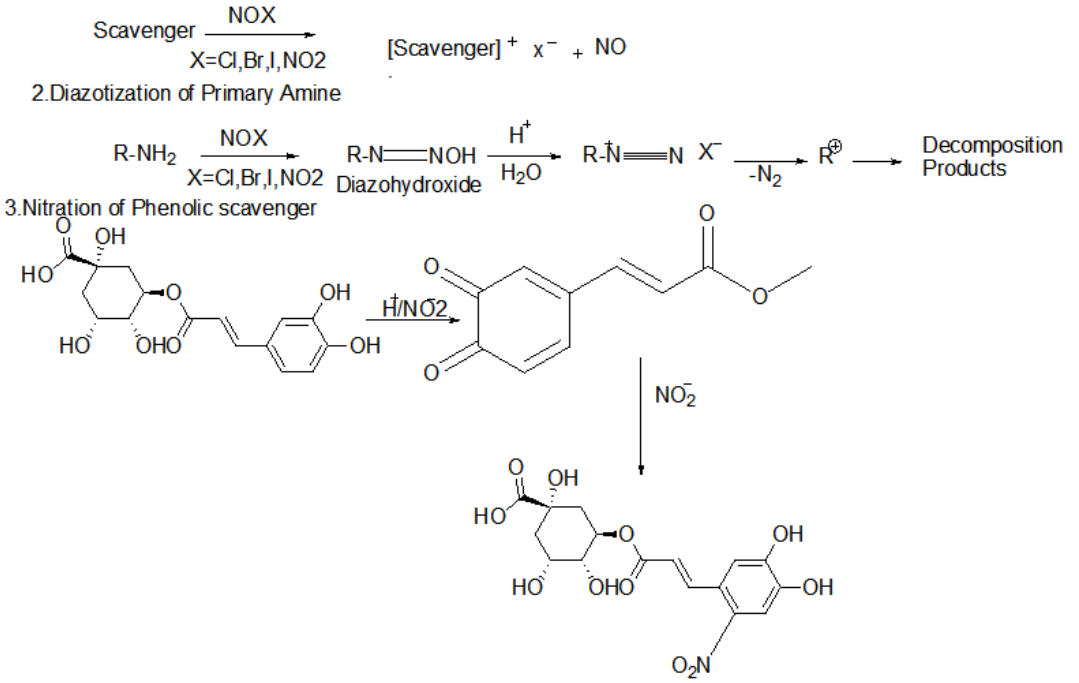

Fig. 7. Proposed Mechanisms of the Main scavenging Pathways of Nitrosating Agents

Table 1. Nitrite scavengers that may be used in solid oral

Nitrite Scavenger	Solubility	Dosage Forms	Key Characteristics
á-Tocopherol	Fat	Tablets, Capsules, Solution, Suspension	Efficient absorption, effective nutrient and nitrite scavenger
Ascorbic Acid	Water	Tablets, Capsules, Suspension, Solution, Syrup, Powder	Versatile, enhances absorption, effective nutrient and nitrite scavenger
L-Cysteine	Water	Tablets, Suspension, Capsules	Easy dissolution and absorption, dietary supplement
Glycine	Water	Powder, Solution, Capsules, Suspension, Tablets	Easy dissolution and absorption, dietary supplement
Arginine	Water	Tablets	Easy dissolution and absorption, dietary supplement
Lysine	Water	Intravenous	Easy dissolution and absorption, potential dietary supplement

NO's potential oxidation to nitro-sating agents in aerobic conditions, ascorbic acid remains a potent scavenger, especially in anaerobic settings [51]. A stoichiometric model in drug formulations calculates the necessary ascorbic acid based on anticipated nitrosating agent levels during storage. In solid forms, particle size and distribution affect scavenging efficacy, with wet granulation providing uniform distribution. Research confirms that ascorbic acid effectively prevents nitrosation, significantly reducing N-nitrosamine formation in tablets and other pharmaceuticals [52] .

á-Tocopherol

Preventing N-nitrosamine production depends significantly on the antioxidant ability of á-tocopherol, which reduces nitro-sating agents to non-nitro-sating molecules [53]. The fully substituted aromatic ring of á-tocopherol minimizes C-nitrosation, [54] with its effectiveness relying on the non-esterified form due to radical production at the hydroxyl group during oxidation. In lipophilic media, á-tocopherol works well, also performing in aqueous environments where the molar ratio of tocopherol to nitrite affects NA suppression [55]. Optimal nitrite reduction occurs at pH 2-3, where less than 25% remains after an hour, versus over 95% at pH 5. á-Tocopherol, especially when combined with ascorbic acid, reduces nitrite faster than ã-tocopherol, significantly lowering nitrosamine production in lipid-rich matrices like bacon and tablets [56].

Amino acid

Amino acids with primary amines or thiol groups, except proline, efficiently scavenge nitrites via diazotization reactions, [57,58] producing unstable diazo intermediates that decompose into alcohols and nitrogen through the Van Slyke reaction [59]. L-cysteine effectively inhibits nitrosamine and N-nitrosonornicotine synthesis, outperforming other amino acids like serine, alanine, and proline. Recent screenings indicate variable efficacy with temperature, but L-cysteine's rapid nitrite reduction and scavenging capability, attributed to its thiol group, make it ideal for solid-state tests [60].

Other substances

Substances like resveratrol, BHT, BHA, maltol, and propyl gallate show potential as excipients to reduce nitrosamine formation. Nitrosamine conversion decreased by 40-50% with lysine and glycine, and by up to 90% with histidine at pH 3.0 and 60°C. However, at ambient temperature and pH 3.0, scavenging effectiveness of histidine, arginine, glycine, and lysine was modest. Poorly water-soluble scavengers, such as resveratrol, BHA, and propyl gallate, are unsuitable for liquid applications [50].

Table 2. Temporary threshold standards for nitrosamines in medications

Drug	Acceptable intake NDBA and NDMA (ng/day)	Acceptable intake NDBA and NDMA (ppm)	Maximum daily dose (mg/day)	Acceptable intake DIPNA, EIPNA and NDEA (ng/day)	Acceptable intake NDEA (ppm)	Acceptable intake MNP (ppm)	Acceptable intake MNP (ng/day)
Irbesartan	96	0.32	300	26.5	0.088	-	-
valsartan	96	0.3	320	26.5	0.083	-	-
Losartan	96	0.96	100(US)	26.5	0.27	-	-
		0.64	150(EMA)	26.5	0.177		
Olmesartan	96	2.4	40	26.5	0.66	-	-
Candesartan	96	3.0	32	26.5	0.83	-	-
Eprosartan	96	0.12	800	26.5	0.033	-	-
Azilsartan	96	1.2	80	26.5	0.33	-	-
Telmisartan	96	1.2	80	26.5	0.33	-	-
Metformin	96	0.032	3000	-	-	-	-
Rifampin	-	-	600	-	-	0.16	96

Table 3. Lists the specific HPLC techniques that have been published for nitrosamine impurity detection

Sr. No	Column type	Mobile phase	Injection volume (final Concentration)	Gradient program			Detection wavelength
1	Inertsil ODS-3 Column (150 × 4.6mm, 5um, GL Science, Toyo Japan)	Mobile Phase A: methanol: water (20:80), methanol v/v); Mobile Phase B: methanol: water (75:25 v/v)	20µL, concentration is not reported	Time(min)	Phase A	Phase B	
				0-14	100	0	
				14-15	100-0	0-100	
				15-27	0	100	228nm
				27-28	0-100	0-100	
				28-40	100	0	
				Runtime 40 for gradient elution, NDMA Time spent in retention: 4.5 minutes			
2	Inertsil ODS-3 Column (150 × 4.6mm,5um, GL Science, Toyo japan)	A mobile phase made up of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B)	10µL (100 µM)	Three injections of each sample were made, and the repeatability of the results was established. The retention periods for NDMA and Valsartan were ¼ 7.8 min and 16.3 min, respectively. Gradient elution began at 0% B in 10 min and grew linearly to 100% in 5 min at a flow rate of 1.0 mL/min.			235nm

Table 4. Disclosed limited techniques for nitrosamine impurity detection using LC-MS

Sr. No	Column type	Mobile phase	Injection volume and stock concentration	Program	LOD	LOQ	Iron source voltage	Delustering Potential (V)
1	The phenomenex Luna PFP2 column (Partilcle size:5 µm, pore size :100Å 250 ×4.6 mm)	Mobile phases used(A): Methanol/ formic acid (1000:1v/v) and (B) MilliQ water / Formic acid water (1000:1 v/v) at a flow rate of 0.6mL/min	The volume used was 100 µL, and the concentration of the stock solution was not reported	Gradient: 5% A for 5 min, increase to 30% A over 20 min and hold for 5 min, then increase to 90% A over 10 min and hold for 2 min, returning to initial conditions. Total run time: 60 min.	The following MS parameters are present: corona discharge at 5 lA with a voltage of 4.5 kV, capillary voltage of 14 V, vaporizer temperature of 450 C, gas flow rate of 95 u.a., auxiliary gas flow rate of 56 u.a., and corona temperature of 250 C. 50–500 uma was the mass range at which dimer production was detected. There was no reporting of LOD or LOQ.			
2	Column Eclipse XDB C18 (150 mm x 2.1 mm x 3.5 µm)	Solvent A (2 mM ammonium acetate) and solvent B (100% methanol) were used in the mobile phase at a flow rate of 150 mL/min.	The stock concentration is between 50 and 800 ng/L in 10 mL.	In the solvent gradient program, solvent B increased in percentage terms from 10% to 45% in 4 minutes, then to 100% in 1 minute and held for 5 minutes, and lastly back to 10% in 1 minute. A 2-minute reequilibration period preceded the sample injection.	Using multiple reaction monitoring (MRM) mode and autosampler-equipped ultra-performance liquid chromatography (Thermo, USA), positive electrospray ionization was employed. The ideal ion spray parameters were curtain gas (N2) at 8, ion source gas at 45, and ion spray voltage at 4500 V. The limit of detection (LOD) for NDMA was determined to be 2 mg/L.			

Regulatory considerations regarding the use of tocopherol and ascorbic acid in pharmaceutical products

Global regulatory bodies like FDA, EMA, Pharmaceuticals and Medical Devices agency (PMDA), Health Canada, and others collaborate to minimize nitrosamine contamination in pharmaceuticals due to widespread manufacturing networks and distribution.

Europe

The executive director of the EMA began a review of nitrosamine impurities in chemically synthesized API in September 2019 following the discovery of N-nitrosamine contamination in pharmaceutical goods in Europe. This evaluation was expanded to cover all human pharmaceuticals in 2020. It is up to the producers or Marketing Authorization Holders (MAHs) to determine the

Table 5. Few reported GC-MS methodologies for detecting nitrosamine impurities

Sr. No	Column type	Temperature of heating zone	Heating Program	Injection volume and sample mode
1.	Restek Rtx-624 with guard column (30 m × 0.32 mm I.D., 1.8 µm)	The GC-MS analysis used a 2 mL injection volume at 240°C with a helium flow rate of 1.5 mL/min. The oven was set to 60°C for 2 minutes, then ramped to 240°C at 150°C/min, held for 10 minutes, totaling a 24-minute runtime. The split ratio was 10.0, with ion source and interface temperatures at 230°C and 240°C, respectively, and a detector voltage of 1 kV. NDMA had a retention time of 6.59 minutes in SIM mode.		
2	SH-Stabilwax (30 m × 0.25 mm × 0.50 µm)	The GC-MS analysis used helium as the carrier gas, pressure-controlled, with a temperature ramp: 40°C for 2 minutes, then to 120°C at 10°C/min, and to 230°C at 25°C/min, for a total runtime of 6 minutes. Headspace parameters included a 1 mL sample injection volume, 120°C oven temperature, 125°C sample line temperature, and 130°C transfer line temperature. The GCMS -QP2020NX with HS-20 operated in SIM mode, with a 230°C interface temperature and a 200°C EI source temperature.		

Table 6. Country Specific Risk Mitigation Efforts to Address the Emerging Nitrosamine contamination crises

Country/ Region	Risk Mitigation Effort	Description	Status
United States	Enhanced Regulatory Surveillance	Increased FDA inspections and tighter regulations on nitrosamine levels in pharmaceuticals [79].	Ongoing
European Union	Implementation of ICH M7 Guidelines	Adoption of guidelines for assessing and controlling mutagenic impurities[80].	Implemented
Canada	Mandatory Testing for Nitrosamines	Health Canada requires routine testing of pharmaceuticals for nitrosamine impurities[81].	In Progress
Japan	Stricter GMP Compliance	Enforcement of stricter Good Manufacturing Practices (GMP) to prevent nitrosamine contamination[82].	Ongoing
India	National Task Force on Pharmaceutical Safety	Establishment of a task force to monitor and address nitrosamine risks in drug production[83].	Active
China	Comprehensive Nitrosamine Testing	Nationwide initiative for testing and controlling nitrosamine impurities in pharmaceutical products[84].	Under Development

risk of N-nitrosamine contamination [61]. MAHs must evaluate risk, test for nitrosamine presence, and implement risk mitigation strategies, like adding nitrite scavengers, demonstrating effectiveness through finished product testing.

USA

In response to the finding of N-nitrosamine contamination in pharmaceuticals, the US FDA released Industry Guidance in September 2020. The steps for evaluating and controlling the risk of N-nitrosamine contamination in medications were described in this guidance. This document outlines mitigation techniques and sets timelines for drug makers to complete phases of pending and approved applications, aligning with EMA's three-step procedures [62]. Mitigation techniques for N-nitrosamines include antioxidants like á-tocopherol and ascorbic acid, which effectively inhibit their formation in pharmaceuticals, highlighting their importance in reducing contamination risks [63].

Acceptable intake (AI) of nitrosamine in drugs

The acceptable intake (AI) of nitrosamines in drugs is based on toxicity data to minimize cancer risk, with regulatory agencies setting AI limits using toxicological assessments like TD50 values. AI values determine specific nitrosamine limits for medications, usually in parts per million (ppm). Accurate analytical techniques with appropriate limits of quantification (LOQ) are crucial. The daily limit for nitrosamine pollutants is set at 26.5 nanograms, ensuring overall exposure remains below harmful levels [64].Temporary threshold standards for nitrosamines in medications is given in table 2.

Analytical Technique

Various analytical techniques like Ultra performance liquid chromatography (UPLC), Gas chromatography (GC), Liquid chromatography-tandem mass spectroscopy (LC-MS/MS), and High-pressure liquid chromatography (HPLC) are used to detect and quantify nitrosamine contaminants in ranitidine and sartans, ensuring pharmaceutical safety.

HPLC

In the past decade, HPLC has revolutionized chemistry and pharmaceuticals by effectively separating complex biological mixtures [65]. Numerous HPLC techniques feature in United states Pharmacopoeia (USP) and European Pharmacopoeia (EU). Stationary phases like C18/Phenylhexyl are popular for nitrosamine analysis, with common detectors being Diode array detector (DAD) (230-233 nm) and Ultraviolet detection (UV) (228 nm). Innovations like Chemiluminescence detector (LC-PR-CLD) enhance nitrosamine detection. Valsartan's NDMA detection limits are 0.00085 µg/mL limit of detection (LOD) and 0.00285 µg/mL (LOQ) [66]. Table 3 provides specifics on a number of documented LC-MS techniques for nitrosamine impurity detection.

LC-MS/MS

LC-MS integrates liquid chromatography with mass spectrometry to separate, quantify, and precisely identify complex mixtures, including polar and unstable molecules [67] . Ionization methods include Electrospray ionization (ESI) and Atmosperic pressure chemical ionization (APCI) , crucial for detecting nitrosamine contamination in food, beverages, and pharmaceuticals. LC-MS/MS optimizes parameters like cone voltage and collision energy for precise Quality assurance and control (QA/QC) of nitrosamine impurities. Studies have shown nitrosamine contaminants in medications like valsartan and ranitidine using LC-MS/MS. Researchers identified eight nitrosamine impurities, including NDEA and NDMA, emphasizing the technique's critical role in ensuring pharmaceutical safety and compliance [68]. Some reported LC-MS/MS methods are given in table 4.

GC-QTOF, GC-MS, GC-MS/MS, and GC-MS-Head Space

An essential tool for examining volatile substances and medicinal components is GC-MS. For nitrosamine detection, nitrogen chemiluminescence and nitrogen-phosphorus detectors are preferred [69] . NDMA and other nitrosamines are commonly analyzed in medications like valsartan, using Deutero N-Nitrosodimethylamine (d6-NDMA) as an internal standard. Methods like Quadrupole time-of-flight mass spectrometer (GC-QTOF), Gas chromatography (GC), GC-MS, and GC-MS-Head Space methods detect nitrosamines in ranitidine and sartans. The US FDA used GC-Head Space to identify four nitrosamines in valsartan, with detection limits of 0.01-0.025 ppm. Different sartans were tested for the presence of NDEA and NDMA using the official medicine control

laboratory (OMCL's) LGL Method. The permitted limits for these nitrosamine impurities per 320 mg of API are 0.080 ppm for NDEA and 0.10 ppm for NDMA, according to this procedure [70]. Few reported GC-MS methodologies for detecting nitrosamine impurities are given in table 5.

Non-Chromatographic Method

Various techniques for analyzing NAs include molecularly imprinted polymers with impedimetric sensors (LOD: 0.85 µg/L), chemiluminescence, and UV-photolysis [71]. In the food industry, spectrophotometry, following the Griess reaction, is used for high concentrations of NAs but has limitations due to variability in Nitrate ($NO_{3)}$ and Nitrogen dioxide (NO_2) ion generation during NDEA analysis [72] Reaction-based colorimetric assays, like the Eisenbrand-Preussman reaction, detect nitrite from cleaved nitrosamines but have high detection limits and require large nitrosamine concentrations. Walsh et al. (2005) developed a yeast-based biosensor using genetically modified yeast with a DNA damage reporter system (RAD54-GFP) and cytochrome P450 enzymes, detecting NDMA at 1.6 mg/L. Bui et al. developed a similar system with detection at 3 mg/L. While these methods are useful, they may face challenges in pharmaceutical testing due to high active pharmaceutical ingredient concentrations [73].

Different methods of sample preparation are available for separating nitrosamine impurities

Several methods are used for preparing samples to separate nitrosamine impurities in pharmaceutical products.

Solid-Phase Extraction (SPE)

Utilizes a solid sorbent to selectively retain nitrosamines from the sample matrix, followed by elution for analysis.

Liquid-Liquid Extraction (LLE)

Involves partitioning nitrosamines between two immiscible liquid phases, typically using organic solvents, followed by separation and concentration.

Derivatization followed by Extraction

Converts nitrosamines into more detectable or extractable forms prior to analysis, enhancing sensitivity.

Precipitation Techniques

Involves adding reagents to selectively precipitate nitrosamines for isolation.

Solid-Phase Microextraction (SPME)

Uses a coated fiber to extract and concentrate nitrosamines from the sample matrix [74].

Each method offers unique advantages based on sample complexity and sensitivity. DCM is a common solvent for nitrosamine extraction, selected per study needs.

The effects of nitrosamine contamination and measures for its mitigation

Global ARB recalls due to nitrosamine contamination highlight the need for improved safety measures by manufacturers, regulators, and healthcare professionals.

Risk Evaluation

Preemptive risk assessment for nitrosamine contamination is essential throughout a medicine's shelf life, starting from production. This is particularly important in API production, especially during tetrazole formation in ARB synthesis. Detailed synthetic route information is vital for identifying impurities, though often omitted in ASEAN registrations. Additional precautions include setting impurity limits and managing trace amines in solvents. In September 2019, the EMA requested nitrosamine evaluations for concerning drugs within six months, prioritizing risk management [75]. Tetrazole-containing drugs are high-priority for nitrosamine identification due to patient exposure risks. Risk assessment may prompt process modifications based on lab tests, with a strong understanding of organic chemistry being essential [76,77]. Mass spectrometry is vital for nitrosamine detection, but screening for unknown pollutants can be challenging due to equipment accessibility and affordability issues in GC-MS and LC-MS analysis

Impurity control for nitrosamines

Controlling contaminants in drug ingredients, excipients, and final products complies with ICH quality principles and pharmacopeial standards. Pharmacopeial monographs establish limits for nitrosamine traces, but addressing mutagenic contaminants like nitrosamines remains challenging despite ICH M7 guidelines. Precise analytical techniques are crucial to avoid contamination during nitrosamine analysis in APIs and medicinal products. Toxicity data inform acceptable limits considering future synergistic effects. N-nitroso dialkylamines

analysis may integrate into standard quality control due to carcinogenic concerns, possibly revising monographs. High-risk medications like tetrazole-containing ARBs [78], warrant nitrosamine analysis, though pharmacopeial monographs may not cover all types. Regulatory agencies collaborate with producers to identify nitrosamine sources. Balancing patient carcinogen exposure risks with medication availability is challenging, mitigated by short-term measures to prevent shortages. Healthcare professionals play a critical role in patient communication, emphasizing adherence and exploring alternative therapies during recalls amidst the nitrosamine crisis. Various have implemented specific risk mitigation efforts to tackle the emerging crisis of nitrosamine contamination in pharmaceuticals, (Shown in table 6).

CONCLUSION

N-nitrosamines, known carcinogens found in medications like antidiabetic drugs and Histamine-2 receptor blockers, have prompted stringent testing and mitigation efforts by regulatory bodies. This review highlighted the need for robust detection methods, used of nitrite scavengers, and ongoing challenges in accurately assessing and managing risks. Tetrazole-based drugs and related amine analogs are particularly affected, necessitating adherence to ICH M7 (R1) guidelines and US Pharmacopeia Chapter <1469> for risk assessment and control. Regulatory bodies, including the US FDA, CDSCO, and EMA, are setting limits and requiring precise quantification to address these issues. Present review concluded that to improve safety, proactive risk management, enhanced industry collaboration, and updated regulatory guidelines are essential, along with incorporating country-specific risk mitigation strategies to manage emerging threats and ensure global drug quality.

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