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Nitrosamine impurities in APIs: A Comprehensive Review

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Abstract

Nitrosamine impurities are a class of substances formed from the reaction of a nitroso compound and secondary or tertiary amines. This class of compounds have received attention in the recent past after the revelation of their genotoxic profile. In this context, many batches of API (Active Pharmaceutical Ingredients) supplied to the market were screened for the presence of nitrosamine impurities and recalled due to the presence of genotoxic impurities above the acceptable limit. In this review, we have discussed, the chemistry of nitrosamine impurities, their classification, a possible source for their origin, strategy to control them. A perspective of different regulatory agencies on nitrosamine impurities is also presented. A few case studies which include Valsartan and Metformin are also provided for better understanding. The advancement in analytical techniques for the detection of nitrosamine impurity is also discussed. This review will improve an understanding of pharmaceutical scientists on nitrosamine impurities in pharmaceuticals and thus will find its potential application in academia as well as in industry.

Keywords
Nitrosamine, Mutagenic, Carcinogenic, Sartans, Nitrosating agents.

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1. History of N-nitrosamines

The history of nitrosamine is as old as 1874. This was first observed by the German chemist, Otto N. Witt during his experimental studies wherein he reacted nitrous acid with secondary and tertiary amines to get condensation product. Witt labeled these compound as nitrosamine due to the presence of nitroso group (-N=O) attached to secondary amine.[1] The carcinogenic nature of dimethyl nitrosamine was observed in 1956 when liver tumours in rats were detected by two British scientists, John Barnes and Peter Magee.[2] The root cause for the formation of nitrosamine was found to be the presence of nitro sating agent and this was evident when an increased case of liver cancer was found in Norwegian farm animals. These animals had been fed on herring meal in which sodium nitrite was present as a preservative. The sodium nitrite reacted with dimethylamine in the fish and produced dimethyl nitrosamine which was found to be carcinogenic during the studies carried out in 1950's.

2. Background of Nitrosamine impurities in API

The nitrosamine impurities became a topic of concern among Pharma players in mid-2018. In 2018 the European medicines regulatory network[3a] realized the presence of *N*-nitrosamines in valsartan,[3b] an antihypertensive drug and instituted regulatory actions across the EU which resulted in the recalls of several lots of valsartan from pharmaceutical companies. Among them, *N*-nitrosamines are so potent mutagenic carcinogens that they are referred to as the "cohort of concern" by the International Council for Harmonization 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.[4] The two best-known nitrosamines, *N*-nitroso dimethylamine (NDMA) and *N*-nitrosodiethylamine

(NDEA) have been classified by the International Agency for Research on Cancer (IARC) as possible human (class 2A) carcinogens, but they are also genotoxic.[5]

3. What are nitrosamines?

Nitrosamines are a class of organic compounds represented by general structure R_1R_2N-NO. [5]Chemically, they consist of nitroso moiety attached to dialkylamine. (Figure 1)

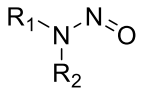


Figure 1: General structure of *N*-Nitrosamine.

4. General root cause for the presence of nitrosamine impurities in API.

The following are the general root cause for the presence of nitrosamine impurity in API.

I. Reaction condition
II. Use of secondary, tertiary, or quaternary amine or their source
III. Contamination in raw materials from the vendor
IV. Recovered solvent, catalyst, and reagents
V. Work up or quenching process
VI. Lack of control on impurity during process optimization
VII. Lack of validated analytical tool for the detection of nitrosamine impurity
VIII. Improperly cleaned reactor
IX. Side reaction of API/API degradation
X. Nitrosamine Impurities in Drug Products during formulation

I. Reaction condition

The main root cause for the formation of nitrosamine impurity is the reaction condition. The reaction condition which uses sodium nitrite under acidic conditions along with secondary, tertiary, or quaternary amines leads to the formation of nitrosamine impurity.[1] (Figure 2) A sequence of reactions is shown in Figure 1 where sodium nitrite first reacts with HCl to form nitrous acid (HNO_2). This HNO_2 upon reaction with dimethylamine leads to the formation of *N*-Nitroso dimethylamine (NDMA).

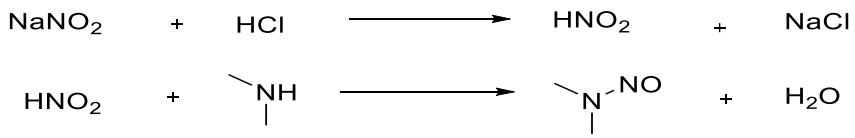


Figure 2: Reaction condition leads to the formation of *N*-nitroso dimethylamine (NDMA) impurity.

The reaction condition which uses nitrite as a reagent is at high risk of generating nitrosamine impurities since nitrites used as reagents in one step can carry over into subsequent steps, despite purification operations/cleaning of the reactor, and react with sec. amines to generate nitrosamine impurities. Therefore, whenever nitrite salts are used in a process, a carryover study should be carried out to ensure its control.

II. Use of secondary, tertiary, or quaternary amine or their source

Secondary, tertiary, or quaternary amines are routinely used in a process for one or another reason which includes their role as a catalyst, or a reagent. These amines upon reaction with nitrous acid or other nitrosating agents lead to the formation of nitrosamine impurities. These amines are also possible in a reaction indirectly from API degradants, contamination in starting materials or use of amide solvents such as *N*, *N*-dimethylformamide, *N*, *N*-dimethylacetamide or *N*-methylpyrollidone. Amide solvents, which are susceptible to degradation under certain reaction conditions, are another source of secondary amines. For example, under high reaction temperatures for an extended reaction period, *N*, *N*-dimethylformamide can degrade into dimethylamine, which can react with nitrous acid to form NDMA. (Figure 3)[7]

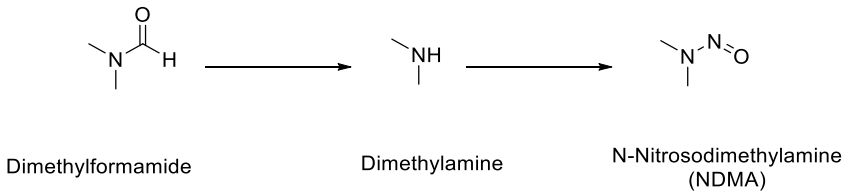


Figure 3: Degradation of *N*, *N*-dimethylformamide (DMF) leads to the formation of NDMA impurity.

Tertiary and quaternary amines are commonly employed in organic synthesis. For example, triethylamine is used as a base in many organic transformations and is known to contain low levels of other secondary amines such as diisopropylamine and isopropylethylamine. Thus, the possibility of the formation of nitroso impurities mainly NDMA and NDEA cannot be ruled out. Similarly, the formation of

nitrosamine impurities are also possible from quaternary ammonium salts such as tetrabutylammonium bromide (TBAB) which are commonly used as a phase transfer catalyst in organic synthesis. In the case of TBAB, there is always the possibility of the formation of *N*-nitrosodibutylamine (NDBA) as shown in Figure 4.[7]

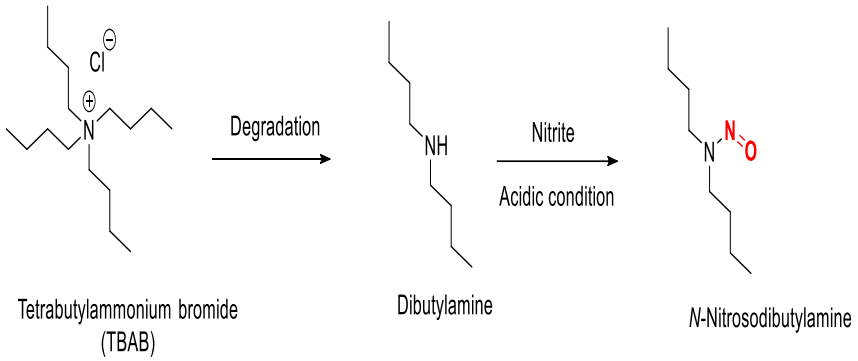


Figure 4: Nitrosamine impurity (NDBA) formation from TBAB, a phase transfer catalyst, commonly employed in organic synthesis.

III. Contamination in raw materials from vendor

Nitrosamine impurities can also be introduced unknowingly from vendor samples/materials including key starting materials, raw materials, solvent, catalyst. The most common observation of contamination in vendors material is presented below.

Ø Nitrosamine contamination in solvents during transfer between storage vessels.

Ø Sodium nitrite contamination in sodium azide is a well-known example of raw material contamination. In this case sodium nitrite is used as a raw material for the synthesis of sodium azide. In this case, the formation of nitrosamine impurity is highly possible when nitrite react with amines under acidic conditions (Figure 5). Similarly, nitrate containing raw materials, such as potassium nitrate, may contaminate with nitrite impurities.

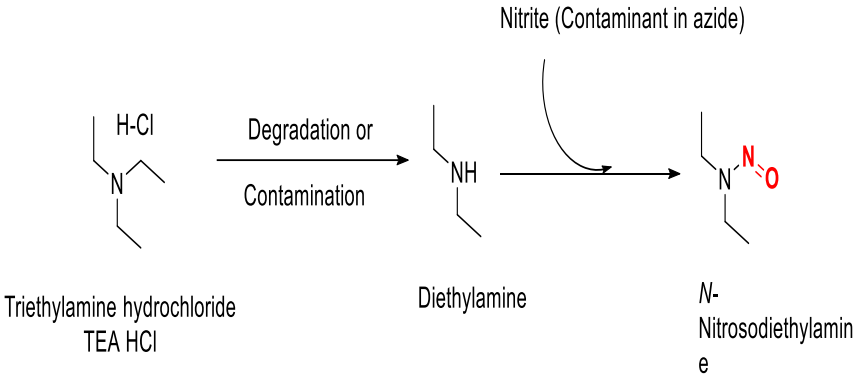


Figure 5: Contamination of sodium azide with sodium nitrite leading to the formation of NDEA impurity.

- Contamination of raw material with secondary or tertiary amines
- Cross-contamination of intermediate/KSM procured with nitrosamine impurities where such impurities are produced.

IV. Recovered Solvents, Catalysts, and Reagents as Sources of Contamination

There are also chances of formation of nitrosamine or contamination of nitrosamine through the recovered solvent, catalyst, and reagents. This happens when residual amines such as triethylamine or diisopropylamine are entrapped in a recovered solvent owing to their similar solubility and/or boiling point profile[8] and further react with nitrous acid coming from the quenching operation. This can be demonstrated with the following examples.

Ø If the recovered solvent, reagents, catalyst are procured from a outside/third party, then also there is a high risk of nitrosamine contamination due to the lack of understanding of nitrosamine impurity/ poor control on the formation of nitrosamine impurity.

Ø It has also been observed that the raw material itself can be contaminated if proper cleaning of equipment is not carried out or is not validated as

capable of removing nitrosamine impurity of concern.

V. Quenching Process as a Source of Nitrosamine Contamination

This is the most common root cause for the formation of nitrosamine impurity. This happen when reaction mass is directly quenched with sodium nitrite and HCl, wherein the amine which is present in a reaction mass is reacted with nitrous acid and thus leads to the formation of nitrosamine impurity. A sequence of reactions is shown below with a classical example of sartan for better understanding.

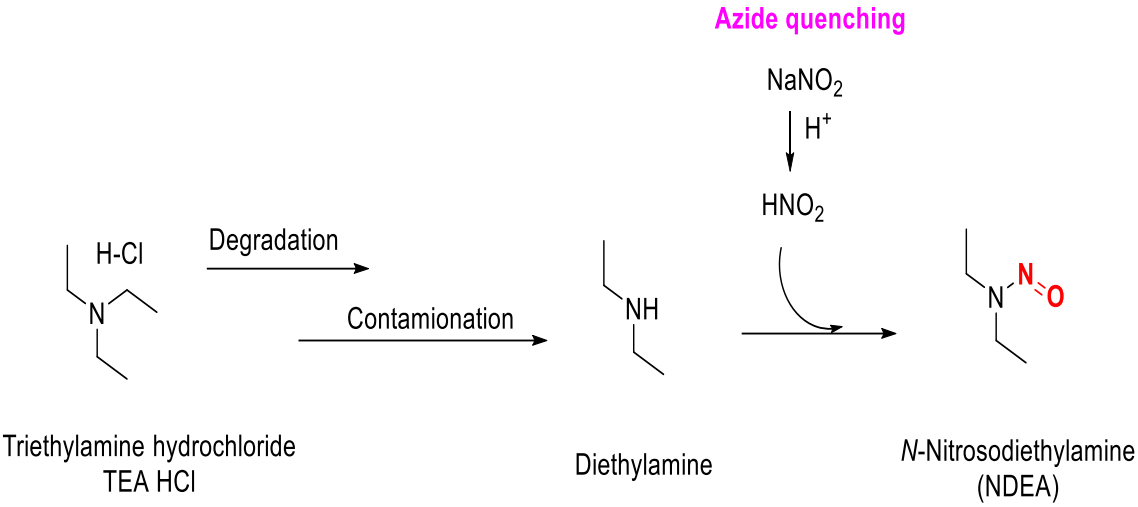


Figure 6: A traditional process for quenching of excess sodium azide present in the reaction mass with sodium nitrite/HCl leads to the formation of NDEA impurity.

VI. Lack of Process Optimization and Control

Another potential root cause for the formation of nitrosamine impurities is the lack of process optimization. This happens due to a poor understanding of the effect of various reaction parameters such as temperature, pH, or the sequence of adding reagents, intermediates, or solvents. When this happens, a variation in batch to batch is observed with respect to nitrosamine impurity.

VII. Lack of validated analytical tool for the detection of nitrosamine impurity

The lack of validated analytical tools can also be a potential root cause for the presence of/ identification of nitrosamine impurity in drug substances. This could be either due to lack of method development or due to improper choice of the analytical instrument.

VIII. Improperly cleaned reactors

This is a common problem that occurs at the plant scale. It has been observed that many a time an improper cleaning of a reactor wherein nitro sating agent is present at traces level reacts with secondary amine present in a reaction mass thus lead to the formation of nitrosamine impurity.

IX. Side reaction of API/ API degradation

The side reactions involving API also lead to the formation of nitrosamine impurities.[9] There are two possible ways for the formation of nitrosamine impurities from APIs; (i) Nitrosation of APIs itself,[9a] (ii) Nitrosation of secondary amine moiety is present as a part of API and subsequent hydrolysis.[9b,9c] Losartan, Valsartan, Irbesartan, Hydrochlorothiazide represent the example where nitrosation of API lead to the formation of Nitroso losartan, Nitroso valsartan, Nitroso irbesartan and 4-nitrohydrochlorthiazide. (Figure 7A). Metformine and Ranitidine represent the case where dimethylamine is a part of a structure which upon degradation and subsequent nitrosation lead to the formation of NDMA impurity (Figure 7B).

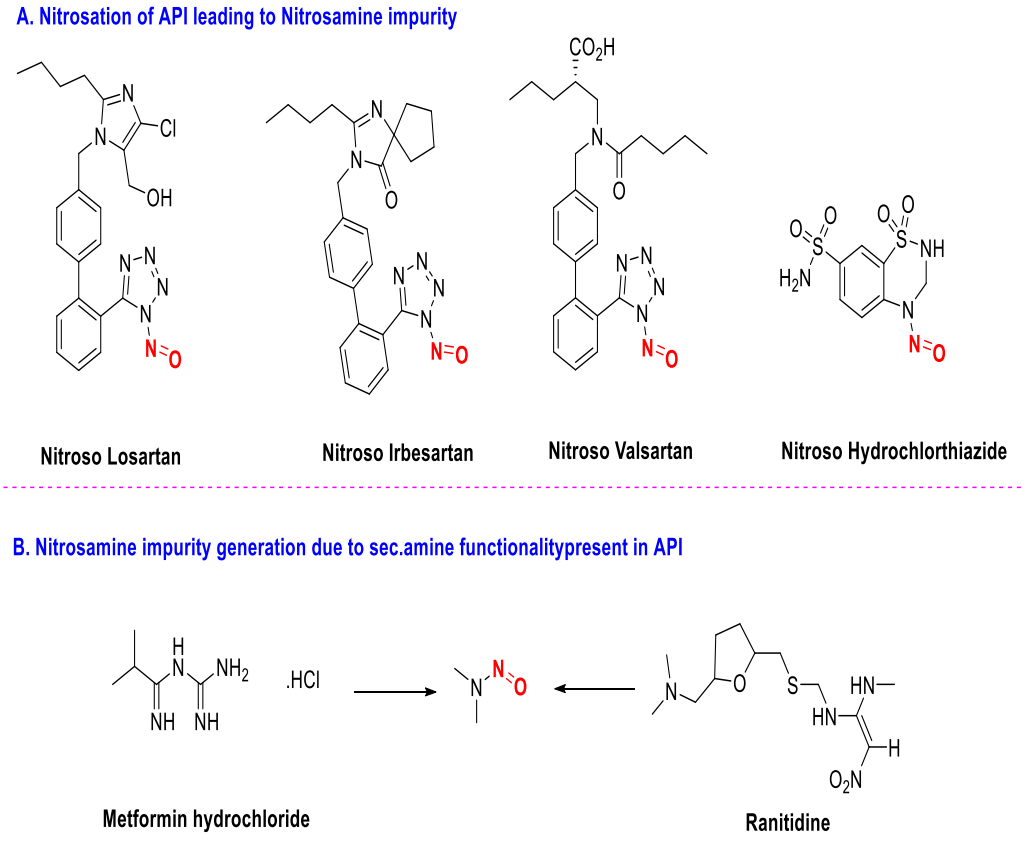


Figure 7: Side reaction of API/ API degradation lead to the formation of Nitrosamine impurity.

X. Nitrosamine Impurities in Drug Products during Formulation

The nitrosamine impurities are not necessarily formed during API manufacturing, they can also be formed during formulations (crystallization, granulation), packaging etc. The packaging material contains nitrocellulose and ink contains amines and thus there is always the possibility of the formation of nitrosamine impurity during packaging.[10]

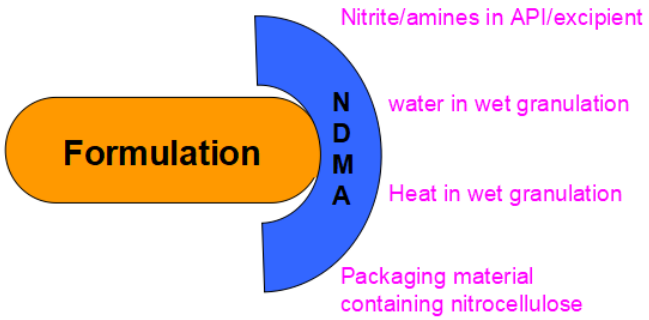


Figure 8: Contributing factors for the formation of nitrosamine impurity (NDMA) in drug product during formulation.

5. Types of nitrosamines

As many as 14 nitrosamine impurities have been reported in the literature. The different nitrosamines are formed from the nitrosation of different amines used in the process for the synthesis of API. A list of 14 different nitrosamines is given in Figure 9. This list can go on increasing upon identification of new nitrosamine impurities.

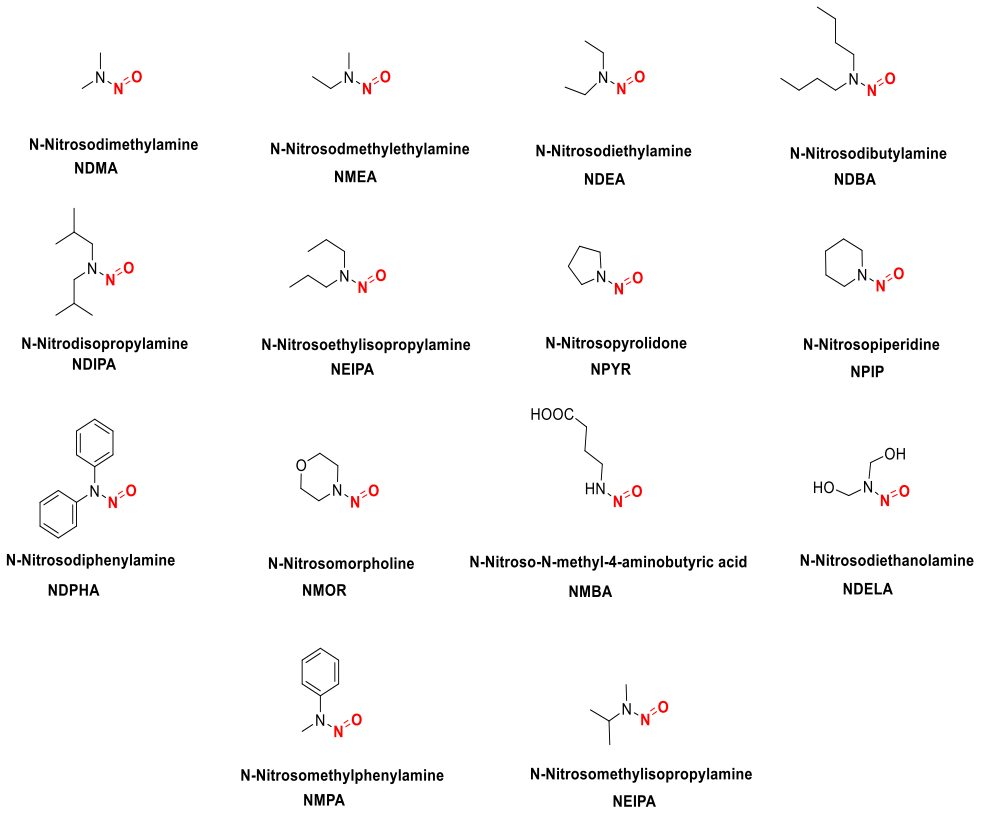
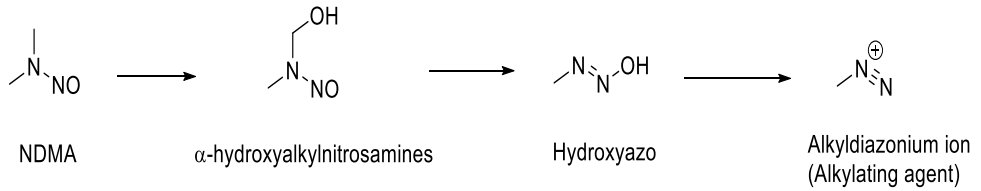


Figure 9: A list of different *N*-nitrosamine impurities reported in the literature.

6. Why Nitrosamines are considered as toxic

Nitrosamine impurities are potent carcinogens and if present in drug substances may pose potential risk hazards. Once *N*-nitrosamines are activated by microsomal liver enzymes, they can react with DNA base pairs to form unstable α -hydroxyalkyl nitrosamines and produce alkyl diazonium ions. This alkyl diazonium ion act as an alkylating agent which alkylates DNA bases and induces carcinogenic response (Scheme 1).[11a-c]



Scheme 1: Proposed reactivity of Nitrosamine with CYP enzyme.

Among them, *N*-nitrosamines are so potent mutagenic carcinogens that they are referred to as the "cohort of concern" by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. The *N*-nitroso dimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA), have been classified under possible human carcinogen by the International Agency for Research on Cancer (IARC).[5]

7. Regulatory perspective

Different regulatory agencies have come forward to issue guidelines on nitrosamine impurities. In this section, we will present a brief overview of the perspective on nitrosamine impurities by different regulatory bodies.

A. European agency

In response to the identification of nitrosamine impurities in the drug products, European Medicine Agency withdrew a series of medicines containing valsartan API manufactured by the Zhejiang Huahai Pharmaceutical Co. Ltd. (ZHP) plant. On 5 July 2018, the European Commission (EC) put the Committee for Medicinal Products for Human Use (CHMP) of the

European Medicines Agency in charge of assessing valsartan medicines in the EU that contained the ZHP-manufactured API, pursuant to Article 31 of the Directive 2001/83/EC on the Community code relating to medicinal products for human use.[12] Subsequently, NDEA i.e. *N*-nitrosodiethylamine, was also found to confirmed in other sartans. Further investigations indicated that *N*-nitrosamine impurity contamination was not limited to the valsartan production at ZHP plant but also affected other sartans containing tetrazole rings.

On 31 January 2019, the Committee for Medicinal Products for Human Use (CHMP) given their view on risk assessment of nitrosamine impurity through a published report. The report suggests that every second person in the Europe is at risk of developing cancer during their lifetime with worst-case scenario of development of cancer to 22 individuals if 100,000 patients took the highest dose (320 mg) of valsartan containing NDMA impurity every day for six years.[13]

After the identification of sources of *N*-nitrosamine contamination, the CHMP recommended short- and long-term measures to fix the problem of nitrosamine impurity in API. In the short-term measure, it set temporary limits for NDMA and NDEA impurities in APIs based on maximum daily intake of 96.0 ng and 26.5 ng, respectively, derived from animal testing and calculated according to ICH M7 (R1) methodology. It was also suggested to consider these limits over a two-year transition period following the Commission's decision adopted on 2 April 2019. It was also highlighted that the responsibility of risk assessment of nitrosamine and control strategy if risk identified are in place lies with marketing authorization holders (MAHs). In the long term, all the API and drug product manufacturer has to change the process which lead to the formation of nitrosamine impurity. [14]

Ranitidine and Metformin were also found to be contaminated with nitrosamine impurities above the acceptable daily intake limit. EMA in September 2019 published another report and recommended to MAHs to extend their risk evaluation to all medicinal products containing a chemically synthesized active substance. [15]

B. USFDA

On 01/09/2021 USFDA issued an update on the control of *N*-nitrosamine impurity for the pharmaceutical industry. This update is to provide guidance on steps to be taken by API and drug product manufacturers to detect and prevent the objectionable level of nitrosamine impurity in pharmaceutical products. The update also described conditions that may introduce nitrosamine impurity.[16]

On 24/02/2021, another update was released by USFDA to ensure the supply of API in the US. The guidance recommends that manufacturers should conclude the risk assessment of approved or marketed products, and follow the mitigation strategy to have control on nitrosamine impurities within 6 months of publication of the guidance. On 18/11/2021, FDA released an update on to provide information to the pharmaceutical industries on possible mitigation strategies to reduce the risk of nitrosamine drug-substance related impurities (NDSRI) in drug products.[16]

In several notifications published by FDA, it has been stated clearly that it is the sole responsibility of API manufacturers to understand the manufacturing process so as to know the possible source for the formation of nitrosamine impurity and their control strategy in intermediate stage itself before they carry over to the final drug substance or drug product. It was also recommended to use validated reliable analytical method for the detection of nitrosamine impurity to avoid any ambiguity about the presence or absence of nitrosamine impurity as per limit set by USFDA. In this context, it is advised to pharmaceutical industries to use methods published by USFDA for the detection of nitrosamine impurity. Parallelly, an acceptable daily intake limits were set for nitrosamine impurities, and it have been made clear that if any lots of drug product were found to contain these nitrosamine impurities above the acceptable limit, then such batches/lot will be recalled by the manufacturer/suppliers.[16]

Daily intake limits set by regulatory agencies

To guide drug substance manufacturer on the limit of nitrosamine impurities, regulatory agencies like the United States Federal Drug Administration (FDA) and European Medicines Agency (EMA) published the acceptable daily intake limits for these nitrosamine impurities.[17a-d] These limits for different nitrosamine impurities are given below.

Ø *N*-nitroso dimethylamine (NDMA): 96 ng/day
Ø *N*-nitrosodiethylamine (NDEA): 26.5 ng/day
Ø *N*-Nitroso-*N*-methyl-4-aminobutyric acid (NMBA): 96 ng/day

The Director of the Center for Drug Evaluation and Research at the US FDA, Dr Janet Woocock, has mentioned that nitrosamine impurities have been found in ranitidine products including Zantac after the identification of these impurities in ARB class of products (sartans). FDA is currently working on evaluation of risk associated with nitrosamine impurity present in ranitidine. FDA is also working on to see the patient taking ranitidine is at risk or not and thus advised to not stop this medication which Is used to treat acid reflux disorder. FDA has also

recommended to consult doctors/physician about the discontinuation of any such medicament.

C. Health Canada

Health Canada has been continuously working on to fix the problem of nitrosamine impurities in sartans above the acceptable level. In order to have better understanding of the problem, health Canada is collaborating with FDA and the EMA in assessing the issue. Meanwhile, it is conducting its own studies on the presence of nitrosamine impurity in ranitidine and assessing the health risk associated with them.[18]

D. The Health Sciences Authority (HSA), Singapore

The Health Sciences Authority (HSA) in Singapore also made an announcement to stop the sale and supply of eight brands of ranitidine products in which NMDA impurities were found to exceed international levels. Aciloc (Uni Drug House), Apo-Ranitidine (Pharmaforte Singapore), Hyzan (Apex Pharma Marketing), Neoceptin (Pharmatec Resouces), Vesyca (Yung Shin Pharmaceutical), Xanidine (Polymedic Trading Enterprise), Zynol (Naina Mohamed & Sons) and three formulations of Zantac (GlaxoSmithKline) are in the list of recalled products.[19]

Table 1: Pharmacopoeial consideration after revelation of nitrosamine impurities in drug products

Sr. No.	API	Pharmacopoeia	Specification wrt. Nitrosamine in monograph	
			Initial	After nitrosamine case revealed
1.	Losartan Potassium	European Pharmacopoeia	No discussion	Following points were discussed about nitrosamine impurity ✓ Presence of nitrosamines in sartan should be avoided or limited as much as possible. ✓ Risk assessment, identification of nitrosamine impurity and process modification to minimize the contamination
2.	Valsartan			
3.	Irbesartan			
4.	Olmesartan			
5.	Condesartan			
6.	Telmesartan			
7.	Azilsartan			
8.	Eprosartan			

After the recalls of several lots of sartan due to the presence of nitrosamine impurities, several guidelines have been issued by regulatory agencies mentioning the root cause and mitigation strategy of nitrosamine impurity in API manufacturing. In the context pharmacopoeia commission also made changes in the monograph.

Central Drug Standard Control Organization (CDSCO), India

The Indian drug regulator, Central Drug Standard Control Organization (CDSCO), is also monitoring updates released by developed countries closely but hasn't come out with any notification on it.[20] The Drugs Controller General of India (DCGI) has urged all the state drug controllers to verify products of drug manufacturers in the context of *N*-nitroso dimethylamine (NDMA) impurity in ranitidine medicine in its circular dated Sep' 19.

7. API contaminated with Nitrosamine Impurity

Ever since the temporary limits were established, nitrosamine impurities have been found in drug products outside the sartans. A list of API which has been reported to be contaminated with nitroso impurity is given below.[21] In this list, sartans stand in the first place.

- Losartan, Valsartan and other ARB (Angiotensin Receptor Blockers)
- Metformin
- Ranitidine
- Rifampin/Rifampicin
- Varenicline
- Pioglitazone
- Nizatidine

The **sartan** class of API is mostly affected among all the API, the main reason could be the tetrazole moiety as a part of API. The synthesis of tetrazole moiety mainly requires azide and amine source and the quenching of reaction mass containing excess azide with sodium nitrite and HCl leads to the formation of nitrosamine impurity. Sartans were the first class of medicines in which nitrosamine impurities were discovered. A modification in the synthetic process for the active pharmaceutical ingredient (API) was identified as the underlying cause of this contamination. Shortly after the valsartan recall, irbesartan and losartan were found to contain NDMA and NDEA. The discovery of nitrosamine impurities in commonly prescribed drugs prompted regulatory authorities to implement a rapid response. The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) published interim limits for several nitrosamine impurities, including NDMA, NDEA and NMBA in sartan products (Table 2).

Table 2: Limit of various nitrosamine impurities in sartans.[21a]

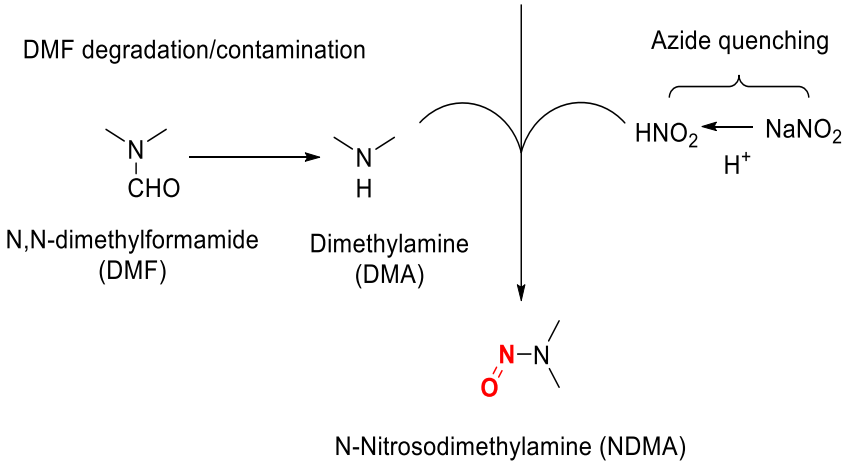
Drug	Maximum daily dose) mg/day)	Acceptable intake NDMA (ng/day) *	Acceptable intake NDMA (ppm)**	Acceptable intake NDEA (ng/day) *	Acceptable intake NDEA (ppm)**	Acceptable intake NMBA (ng/day) *	Acceptable intake NMBA (ppm)**
Valsartan	320	96	0.3	26.5	0.083	96	0.3
Losartan	100	96	0.96	26.5	0.27	96	0.96***
Irbesartan	300	96	0.32	26.5	0.088	96	0.32
Azilsartan	80	96	1.2	26.5	0.33	96	1.2
Olmesartan	40	96	2.4	26.5	0.66	96	2.4
Eprosartan	800	96	0.12	26.5	0.033	96	0.12
Condesartan	32	96	3.0	26.5	0.83	96	3.0
Telmisartan	80	96	1.2	26.5	0.33	96	1.2

*The acceptable intake is a daily exposure to a compound such as NDMA, NDEA, or NMBA that approximates 1 1:100,000 cancer risk after 70 years exposure.
** These values are based on a drugs maximum daily dose as reflected in the drug label
*** FDA is temporarily not objecting to losartan with NMBA below 9.82 ppm remaining on the market.

8. Case studies

I. Valsartan

A contamination due to nitrosamine impurities was observed in valsartan manufactured by modified process at the Zhejiang Huahai Pharmaceutical Co. Ltd. (ZHP) plant after July 2012. The main reason to modify the process was to improve yield and reduce waste. The change includes the replacement of tributyltin azide with a more toxic sodium azide and use of *N*, *N*-dimethylformamide (DMF) as the solvent. Sodium nitrite, which forms nitrous acid in an acidic medium, was used to quench the excess sodium azide, which resulted in the nitrosation of dimethylamine impurity present in *N*, *N*-dimethylformamide to form NDMA (Scheme 2).



Scheme 2: The root cause of formation of Nitrosamine impurity (NDMA) in the route of synthesis of Valsartans by ZHP plant.

II. Metformin

In December 2019, FDA became aware that some lots/batches of drug product containing metformin were reported to contain NDMA impurity.[22] Upon investigation on the presence of nitrosamine impurity in metformin tablets, it was found that there are two main sources for the presence of nitrosamine impurity in the tablet, (i) During manufacturing of API, (ii) during the formulation of API (Figure 10).

The manufacturing process for the preparation of metformin involves the reaction of dimethylamine hydrochloride with cyanoguanidine in a water medium. The dimethylamine hydrochloride on contact with traces of nitrite present in water leads to the formation of NDMA impurity.

In a recent study to investigate the root cause for the formation of nitrosamine impurity in metformin tablets, it was claimed that NDMA impurity is also formed during formulation and the contributing

factors are water, heat, and excipient containing nitrite used for the granulation or tablet manufacturing.

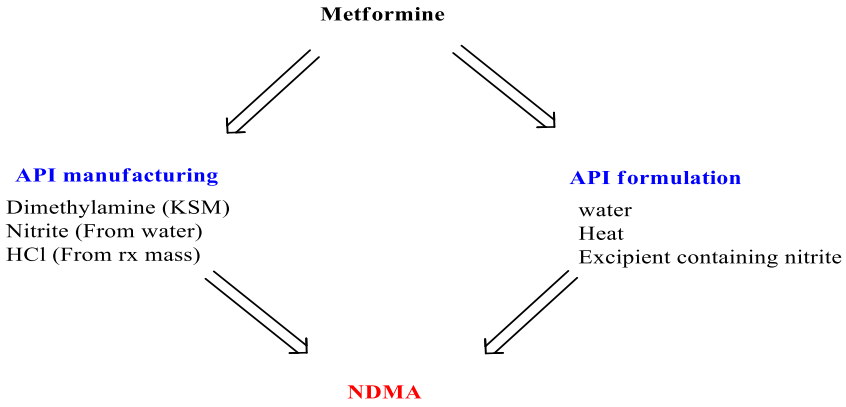
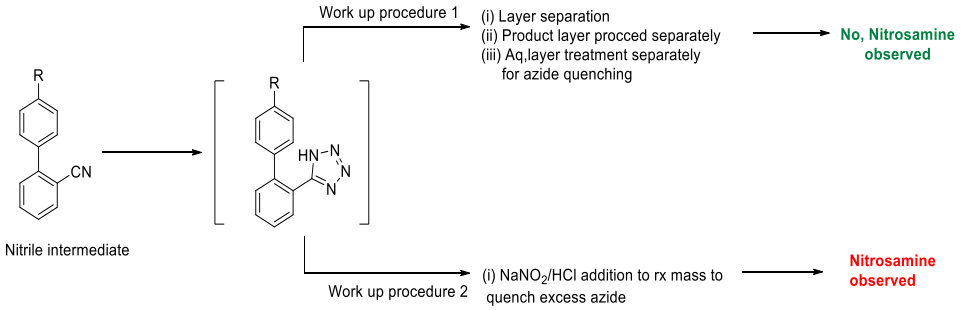


Figure 10: Root cause for the formation of NDMA impurity in metformin

9. Control strategy/Mitigation strategy

In order to have control over the presence of nitrosamine impurity in drug substances one requires a deep scientific understanding of how nitrosamines can form and purge in an API manufacturing process. It is the responsibility of process development scientists to have the best possible understanding to evaluate and address risks. This can be done by carefully reviewing the chemical steps involve in the synthesis of API and identifying the raw materials/recovered solvents used for the synthesis and formulation of API. This will help them to design controls to minimize the risk from nitrosamine. This is demonstrated in the following example.



Scheme 3: A schematic diagram showing control of nitrosamine impurity by a change in workup procedure.

It is being recommended by the drug regulator authority to follow ICH M7 guidelines which talk about the control of mutagenic impurities in the drug manufacturing process.[23] It is also advised to consider the ICH Q9 guideline on quality risk management and adopt Good Manufacturing Practice (GMP). This provides a framework to identify, assess and develop controls that should be used for nitrosamines. This framework requires the application of scientific principles by process development scientists and review of the manufacturing process including all plant operations by regulators through inspection.

The FDA updated the guidance in February 2021 to provide mitigation activities by drug manufacturers. This is a three-step strategy which includes risk assessment, as step 1, confirmatory testing if risks are identified, as step 2, and reporting changes implemented to prevent or reduce the presence of nitrosamine impurities in drug products, as step 3

10. Analytical techniques for the detection of Nitrosamine impurity

A lot of development has happened in the field of analytical chemistry toward the development of a method for the detection of different nitrosamine impurities present in API. Buldini *et al*. in 1987, reported a voltammetric method for the detection of 1-nitroso-lH-hexahydroazepine impurity in antidiabetic drug, Tolazamide. Owing the low sensitivity of traditional analytical techniques for the detection of nitrosamine impurities, development of advance analytical methods like LC-MS, GC-MS, SCF,

LC-HRMS took place. In this section, we will review, analytical techniques developed for the detection of nitrosamine impurity/impurities (Table 3).

Table 3: List of analytical methods reported for the detection of various nitrosamine impurities.

Year	Authors	Analytical methods	Description/advantage	Nitrosamine	API
1987	Buldini et al.[24]	Voltammetric	Detection of both volatile and nonvolatile compound	1-nitroso-lH-hexahydroazepine (NHHA)	Tolazamide
2019	Fritz Sörgel [25]	LC-MS/MS with APCI ionization	Sensitivity	NDMA, NDEA	Valsartan
2019	Sebastian Schmidtsdorff [26]	supercritical fluid chromatography	Detection of large class of different nitrosamines in the ppb range	NDMA, NDEA, NDIPA	Sartans
2019	Sayaka Masada et al. [27]	HPLC	Rapid and efficient	NDMA	Valsartan
2020	Lim et. Al[28]	GC tandem mass spectrometry	Sensitive, accurate, Simultaneous detection of NDMA and NDEA	NDMA, NDEA	Sartans, Ranitidine Metformin
2020	Jingyue Yang[29]	Quantitative LC-HRMS		NDMA, NDEA, NEIPA, NDIPA, NDPA, NMPA, NDBA, NMBA	Metformin
2020	Venkatesan et al.[30]	GC-MS	Cost effective, accurate and precise	NDMA, NDEA	Olmesartan medoxomil
2021	Sebastian Schmidtsdorff [31]	SFC-MS/MS		NDMA, NMEA, NDEA, NDELA, NEIPA, NDIPA, NDPA, NDBA, NMPhA, NMEPhA, NDPhA, Npyr, Npip, NMor, MNPaz, NMBA.	Sartans, Metformin, Pioglitazone Ranitidine
2021	Shu-Han Chang[32]	GC-MS/MS	Sensitive and effective	NDMA, NDEA, NDPA, NMOR, and NPIP	Sartans
2021	Chidella et al. [33]	LC-MS/MS	Ultra-sensitive with LOQ 0.004 ppm	NDMA, NDEA, NEIPA, NMBA, NDIPA and NDBA	Telmisartan
2021	Campillo et al.[34]	GC-MS	Simple and sensitive	NEMA, NDEA, NPYR, NMOR, NDPA, NDMA, NDBA and NDPA	Ranitidine
2021	Wichitnithad [35]	Headspace GC-MS	LOD as low as 5 ppb	NDMA, NDEA, DIPNA, EIPNA	Losartan
2021	Liu et al.[36]	GC-MS/MS	Sensitive and stable, LOD 0.002 ppm	NDMA, NDEA, NEBA, NDIPA	Sartans
2021	Xu et al.[37]	Single quadrupole LC/MS	LOD 0.05 ppm, large injection volume	NDMA, NDEA, NDIPA, NEIPA, NDBA, and NMBA	Valsartan

11. FUTURE PERSPECTIVE

The problem of nitrosamine contamination is not just limited to sartans or a few selected APIs but can be extrapolated to other API as well. With the advancement of analytical techniques or more vigorous screening of API for nitrosamine impurity, there are high chances for the detection of many new types of nitrosamine impurity in API. Thus all scientific community is required to work in collaboration to minimize the risk. In this direction all pharmaceutical professionals such as process development scientists, analytical scientists, process engineers, chemical engineers and formulation scientists to be vigilant from their end to overcome the possibility of nitrosamine impurity in drug substances or drug products to avoid health hazard to human beings.

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