Guidance on Nitrosamine Impurities

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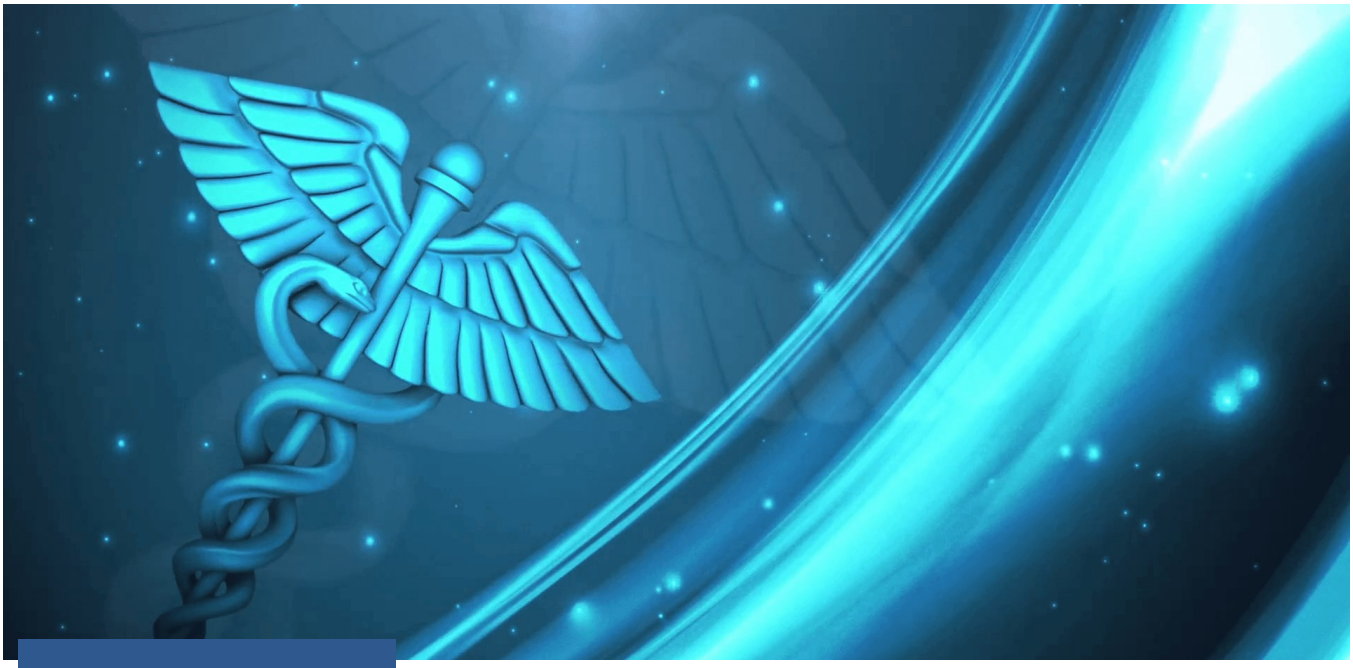


PREFACE

In April 2015, The IPA launched its Quality Forum (QF) to help Indian pharmaceutical manufacturers to achieve parity with global benchmarks in quality. The QF made a commitment to a multi-year journey to address key issues facing the industry and develop best practices.

The QF focused on several priority areas in the last four years, namely, Data Reliability, Best Practices & Metrics, Culture & Capability, Investigations, etc. It took upon itself the challenge of developing a comprehensive set of Best Practices Documents for several of these topics. In this document, we focus on best practices for Nitrosamine and other ICH M7 impurities. We had released a comprehensive set of Data Reliability Guideline in February 2017, Process Validation Guideline and Good Documentation Practice Guideline in February 2018, Investigation of non-conformities in February 2019 and Handling Market Complaints Best Practices in February 2020.

The six participating companies in the QF nominated senior managers to study the best practices and frame the guidelines. They are: Vijay Shanbhag (Cipla); B M Rao (Dr Reddy's); B N V Ganpati Rao (Dr Reddy's); Rajiv Desai (Lupin); Nalin Karkra (Sun); Priti Shah (Torrent); Gunvantsinh Desai (Zydus Lifesciences) and Ramreddy Chandireddy (Zydus Lifesciences). The IPA wishes to acknowledge their concerted effort over the last 12 months. They shared current practices, benchmarked these with the existing regulatory guidance from the USFDA and other regulatory bodies such as UKMHRA, WHO, etc., developed a robust draft document and got it vetted by a leading subject matter expert and regulatory agencies. The IPA acknowledges their hard work and commitment to quality.

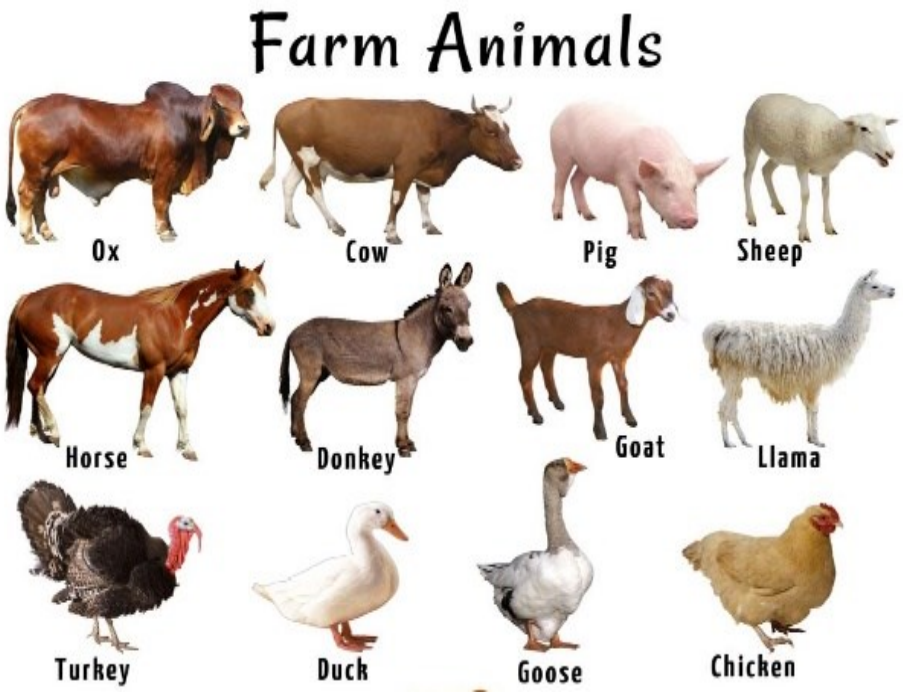
The IPA also wishes to acknowledge the CEOs of six member-companies who have committed their personal time, human resources and provided funding for this initiative.

This document, to be released at the IPA's Advanced GMP Workshop 2022, will be hosted on the IPA website www.ipa-india.org to make it accessible to all manufacturers in India and abroad.

1 Introduction

1.1 Background of Nitrosamines:

- The synthesis of Active drug substances involves the use of reactive chemicals, reagents, solvents, catalysts and other processing aids. Because of chemical synthesis, side reactions or subsequent degradation, impurities residue may present in all drug substances and associated drug product. The philosophy is fundamental for the control of impurities such guideline, ICH M7 (R1), which complements others such as ICH Q3A, ICH Q3B, ICH Q3C, ICH Q3D and ICH9 provides information on N-Nitrosamine(s) which are considered part of a 'cohort of concern' because of their mutagenic potency.
- In 1956, two British scientists, John Barnes and Peter Magee, reported that dimethyl nitrosamine, produced liver tumours in rats. They discovered this during the routine screening of chemicals that were proposed for use as solvents in the dry chemical industry. Subsequent studies showed that approximately 90% of the 300 nitrosamines tested were carcinogenic in a wide variety of animals.



- The World Health Organization has classified nitrosamines as carcinogenic to humans.
- N-Nitrosamines are a class of compounds characterized by the binding of a nitroso group (-N=O) to an amine functional group (-NR2). Among the compounds of this class are some agents mutagenic, genotoxic and potentially carcinogenic in humans, therefore, should be controlled to levels considered acceptable and safe.
- These compounds can be commonly found in water, in animals, in smoked and grilled foods, dairy products, as well as alcoholic beverages and vegetables and their exposure within safe limits represents a low risk of health problems. However, exposure above acceptable levels and for long period may increase the risk of cancer.

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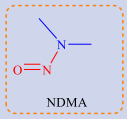
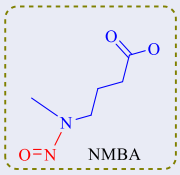
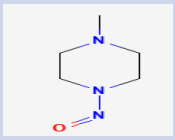
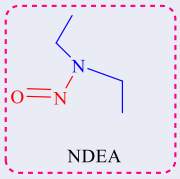
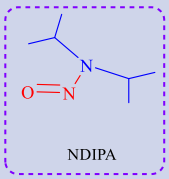
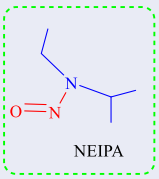
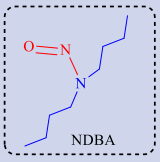
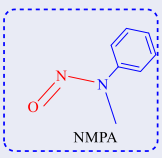
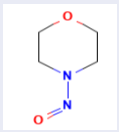
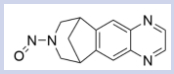
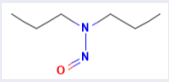
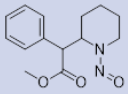
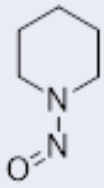
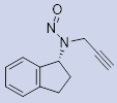
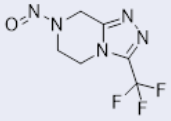
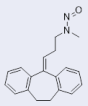
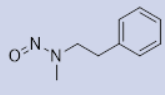
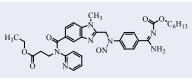
- Below are the nitrosamine impurities identified in the EMAs guideline for nitrosamine impurities as of July 2022, here lowest Acceptable Intake (AI) are considered, however there are differences in AI limits between agencies.

Name of Impurity	Code	Chemical Formulae	Molecular Weight	CAS No.	Structure	Acceptable Intake (AI) (ng/day)	Boiling/ Melting Point	Solubility
N-Nitrosodimethylamine	NDMA	$C_2H_6N_2O$	74.08	62-75-9	 NDMA	96	B.P. 153 °C	Soluble in Water, Ether & Ethanol
N-Nitroso-4-(methylamino)butyric acid / N-Nitroso-N-methyl-4-aminobutyric acid	NMBA	$C_5H_{10}N_2O_3$	146.15	61445-55-4	 NMBA	96	M.P. 33 - 34°C	Chloroform (Soluble), Dichloromethane (Slightly), Ethyl Acetate (Slightly)
1-Methyl-4-nitrosopiperazine	MNP/ MeNP	$C_5H_{11}N_3O$	129.16	16339-07-4	 MNP	26.5		Chloroform (Slightly), Ethyl Acetate (Slightly)
N-Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102.14	55-18-5	 NDEA	26.5	B.P. 177 °C	Completely Soluble in Water, Very Soluble in Ether, Alcohol.
N-Nitrosodiisopropylamine	NDIPA/ DIPNA	$C_6H_{14}N_2O$	130.19	601-77-4	 NDIPA	26.5	M.P. >300°C	Benzene (Slightly), Chloroform (Slightly), Methanol (Slightly)
N-Nitrosoethylisopropylamine	NEIPA/ NIPEA/ EIPNA	$C_5H_{12}N_2O$	116.16	16339-04-1	 NEIPA	26.5	--	Methanol (Soluble)
N-Nitrosodibutylamine / N-Nitroso-di-n-butylamine	NDBA	$C_8H_{18}N_2O$	158.25	924-16-3	 NDBA	26.5	B.P. 237 °C	Soluble in Acetone, Dichloromethane and Ethyl Acetate.
Nitrosomethylphenylamine / N-Nitroso-N-methylaniline	NMPA	$C_7H_8N_2O$	136.15	614-00-6	 NMPA	26.5 (US FDA)	M.P. 13 °C B.P. 128 °C/19 mmHg	In soluble in Water, Soluble in Ethanol and Ether.

Name of Impurity	Code	Chemical Formulae	Molecular Weight	CAS No.	Structure	Acceptable Intake (AI) (ng/day)	Boiling/ Melting Point	Solubility
N-Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116.12	59-89-2	 NMOR	127	M.P. 29 °C. B.P. 225 °C	Completely soluble in Water. Soluble in Methanol.
N-Nitrosovarenicline	NNV	$C_{13}H_{12}N_4O$	240.26	2755871-02-2	 NNV	37.0	M.P. >169°C	Chloroform (Slightly), Methanol (Slightly)
N-Nitrosodipropylamine	NDPA	$C_6H_{14}N_2O$	130.19	621-64-7	 NDPA	26.5	B.P. 113 °C/40 mmHg	Soluble in Water
N-Nitrosomethylphenidate	NMPH	$C_{14}H_{18}N_2O_3$	262.30	55557-03-4		1300		
N-Nitrosopiperidine	--	$C_5H_{10}N_2O$	114.15	100-75-4		1300		Chloroform (Slightly), Ethyl Acetate (Slightly), Methanol (Slightly)
N-Nitrosorasagilene	--	$C_{12}H_{12}N_2O$	200.2	2470278-90-9		18		
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine	--	$C_6H_6F_3N_5O$	221.14	NA		37		
N-Nitroso-1,2,3,6-tetrahydropyridine	NTHP	$C_5H_8N_2O$	112.13	55556-92-8		37		
N-Nitrosonortriptyline	--	$C_{19}H_{20}N_2O$	292.4	55855-42-0		8		
N-Methyl-N-nitrosophenethylamine	NMPEA	$C_9H_{12}N_2O$	164.2	13256-11-6		8		
N-Nitro dabigatran	-	$C_{34}H_{40}N_8O_6$	656.53	-		18		

- Since the year of 2018, regulatory agencies around the world became aware of the presence of nitrosamines above permitted levels in medicines, after manufacturers of active pharmaceutical ingredients of the group of drugs commonly called "sartans" and several drug products including ARBs, ranitidine, nizatidine, and metformin have been found to contain unacceptable levels of nitrosamines.

- Through investigation, the agency determined that numerous lots of valsartan and a few other ARB drug products from different manufacturers contained unacceptable levels of nitrosamines. The drug product manufacturers voluntarily recalled the affected batches of these drug products, which led to a drug shortage in some of the affected products. In addition, FDA evaluated processes that use common amines in API synthesis and learned that common synthetic pathways could also introduce other types of nitrosamine impurities besides NDMA.

- Since then, these agencies have action taken to protect the health of patient's exposure to nitrosamines in drugs above levels considered acceptable. In Brazil, ANVISA withdrew approximately 200 batches of medicines of Sartans type. Besides, the agency suspended the manufacturing, import, supply, marketing and use of APIs with suspect of contamination. In total, were made 14 suspensions of three ingredients. The source of contamination of the medicines was identified as arising mainly from the presence of solvents under chemical conditions that favour the formation of nitrosamines. Additionally, there were evidences of possible formation of nitrosamines from primary packaging material containing nitrocellulose.

- Faced with the case of the "Sartans", the main regulatory agencies in the world, together with drug companies began to investigate whether other drugs could also contain nitrosamines above acceptable levels. In September 2019, it was known that some common heartburn products (Ranitidine, commonly known as Zantac, and Nizatidine, commonly known as Axid) contained unacceptable levels of NDMA.

- As part of this investigation process, it was found, for example, that the presence of nitrosamines in ranitidine had a different source from that previously found in other products. For this drug, the formation of N-Nitrosodimethylamine (NDMA) originates from an intermolecular degradation, which occurs throughout the storage of the product and is accelerated by storage at temperatures higher than room temperature. Such conditions can result in consumer exposure to levels unacceptable effects of this impurity. There is also evidence that the rate of formation of this contaminant is linked to the particular crystal morphology of the molecule.

- In September 2019, at the request of the European Commission, an Article 31 review was initiated for ranitidine containing medicines (EMEA/H/A-31/1491) after tests showed that some of these products contained NDMA, both in API and finished products. In several EU countries, national authorities initiated recalls of ranitidine medicines from pharmacies. It is also noteworthy that medicines containing ranitidine hydrochloride have been available to the world population for over 30 years and are used to treat ulcers, esophagitis and gastric reflux, without notification of serious adverse events, which reinforces the need for evaluation and control of nitrosamines in all drug classes.

- It is important to emphasize that although there is a very low risk of nitrosamines being present in biological products, these cannot be definitively discarded. In light of knowledge scientific research, it is known that such risks are concentrated, for example, in products with chemically synthesized fragments, those packed in blisters containing nitrocellulose, biological products with excipients in its composition, or where there is the intentional addition of nitrosating agents in the manufacturing process.

- Despite available guidance, the potential for N-nitrosamine impurities in sartans was not recognised during the development, manufacture and evaluation of medicines subsequently found to contain them. In addition, MAHs, who usually outsource API manufacturing, may not have had enough oversight of the manufacturing processes.

- The lessons learnt group evaluated relevant guidelines and determined that certain amendments and clarifications would help both companies and regulators had better assess the potential for impurities such as N-nitrosamines. In addition, the group proposed further training for regulatory assessors in the network to improve the chances of identifying potential impurities during the evaluation of marketing authorisation applications and certificates of suitability (CEPs).

- The network reacted swiftly once the presence of N-nitrosamines became known, taking immediate measures to protect patients and the quality of medicines in Market. These measures included coordinating recalls of medicines across the market, prohibiting the use of affected APIs [via CEP suspensions or issuance of certificates of non-compliance with good manufacturing practice (GMP)], testing of medicines on the market, inspecting manufacturing sites and conducting an EU-wide review of sartans medicines.

- As the nitrosamine impurity issue extends, FDA and other regulatory authorities have collaborated to share information, coordinate inspection efforts, communicate effective analytical methods to detect and identify various nitrosamines, and to develop rapid solutions to ensure the safety and quality of the drug supply.

- The lessons learnt group concluded that public communication from regulators could be improved by including in their communication materials more specific details such as batch numbers (e.g., following recalls) and available alternatives. Other ways to improve the impact of public communication include working more closely within the network, using more tools such as social media, and taking extra measures to reach target audiences.

- This presents recommendations for the control of nitrosamines in medications as well how it clarifies the responsibility of companies, presents strategies for calculating limits and addresses other concepts.

- However, the pharmaceutical industry, are encouraged to conduct their own exercises and consider what additional actions they should take.

1.2 Origin of Impurities:

- In acidic conditions, secondary or tertiary amines react with nitrites to form nitrosamines. There are a number of pathways by which nitrosamines can be introduced into or generated as impurities in pharmaceutical drug products.

    - API processing done under specific conditions and in the presence of certain reagents, solvents raw materials and processing aids, there is evidence that despite processing and purification steps, reactive species, whether intentionally added to or formed during the process reaction sequences, (e.g., nitrites and secondary amines in the presence of acidic conditions), special attention should be given to the formation of nitrogen containing heterocycles by employing azide followed by quenching with nitrite to remove excess azide.

    - The API itself, which may degrade under some conditions resulting in the formation of nitrosamines (e.g., ranitidine).

    - Degradation of solvents (e.g., dimethylformamide [DMF]) leading to the formation of dialkyl amines.

    - Impurities in raw materials, solvents (including recycled solvents), reagents, or catalysts.

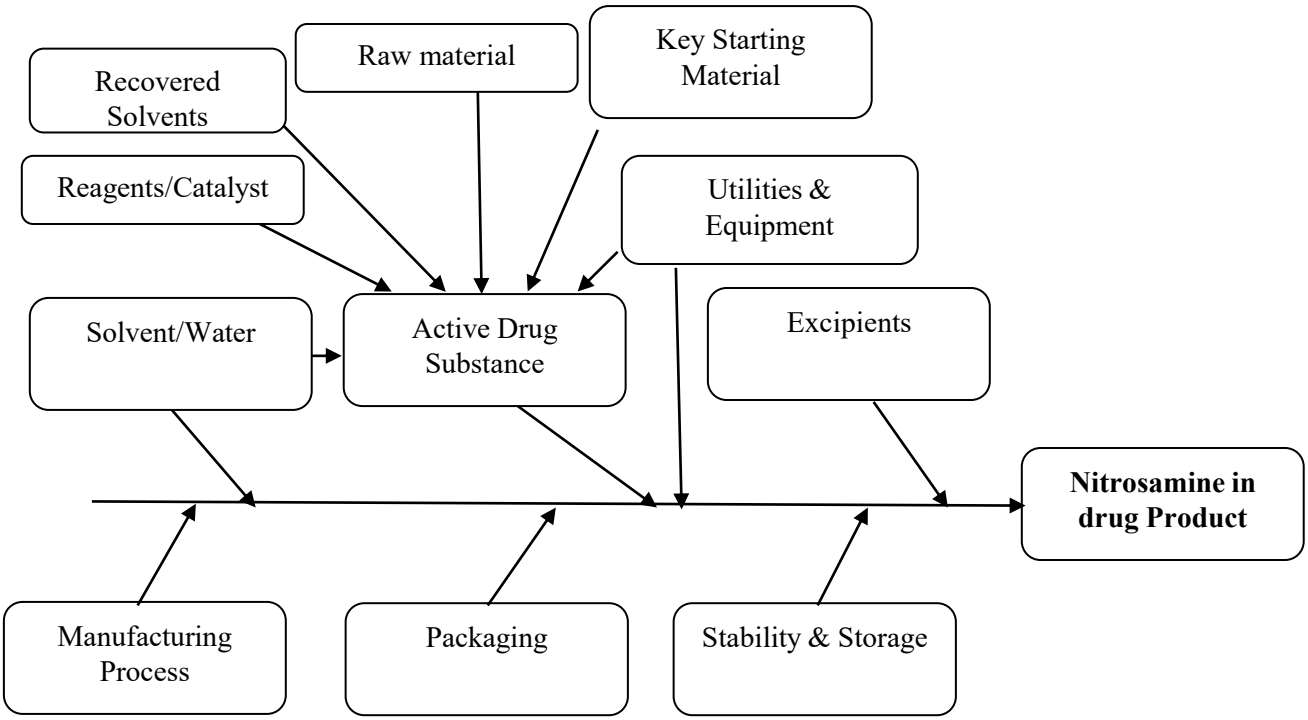
    - Impurities in materials and intermediates, reagents, and solvents used to prepare the starting materials or intermediates.

    - Impurities in water, excipients, or processing aids used in the production of the finished drug products.

    - During drug product manufacture under certain reaction conditions and in the presence of requisite precursors necessary for the formation of nitrosamines.

    - Impurities in the container closure system for the finished drug product which may include impurities capable of forming nitrosamine specially if associated with materials containing amines and potential sources of nitrosating agents (e.g., nitrite, nitrocellulose).

    - A risk assessment should be conducted to determine the materials that contribute to the potential for inclusion of nitrosamines in the drug product. All potential sources for the introduction of nitrosamines should be considered in the risk assessment including, for example, the drug substance, excipients, water, solvents, the manufacturing process, packaging components, and formation on stability. Below diagram of some potential source to be considered in Risk assessment.

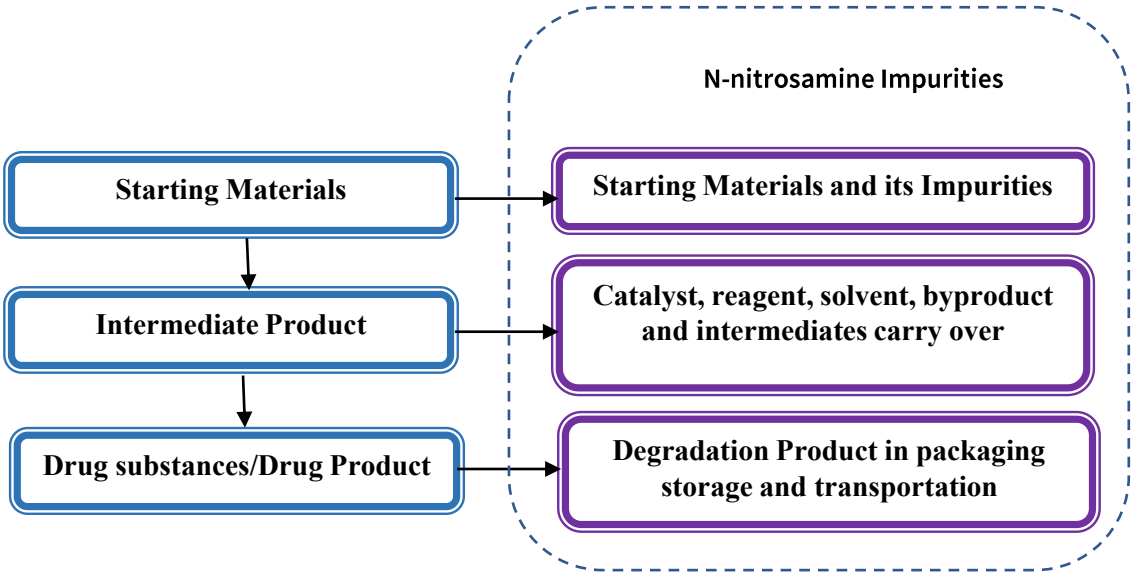


Potential Source of Nitrosamine Impurity in Drug product.

- On going assessments evaluations have identified risk associated with Several of the potential sources of the nitrosamine, below are the few examples summarised.

Potential Sources Of Impurities	Observed Risk
Solvents	❖ Presence of residual dialkyl amines or tri-substituted amines that can degrade to form dialkyl amines (e.g., triethylamine). ❖ Presence of nitrites or other nitrosating agents ❖ Presence of acid ❖ Limited controls/specification limits for recycled solvents. ❖ Poor Quality water or solvents
Water	❖ Presence of residual dialkylamines amines or impurities that can degrade to form dialkylamines ❖ Presence of nitrites or other nitrosating agents in presence of acid.
Excipients	❖ Presence of nitrites or other nitrosating agents
Drug substance	❖ Use of sodium azide and sodium nitrite for azide quenching in the synthesis in acid media ❖ Use of di- or tri-alkylamines and amides (e.g., dimethylformamide [DMF], dimethylamine [DMA], triethylamine [TEA], N-methyl pyrrolidone [NMP]) in the presence of nitrites and acid media ❖ Use of recycled solvents that may contain nitrosamines or their precursors ❖ Use of sanitized water (e.g., chloramines) ❖ Need of additional purification steps (Crystallization)
Manufacturing process	❖ Contamination ❖ Use of recycled solvents that may contain nitrosamines or their precursors ❖ Poor quality solvents ❖ Presence of nitrous oxides in air used to dry the API or drug product
Drug product (including stability)	❖ Secondary or tertiary amine group in molecule in presence of nitrite counter ions (potentially as an impurity) ❖ Potential reactions within the formulation matrix during stability/shelf life (e.g., presence or generation of acidic conditions, moisture, and heat).
Container–Closures	❖ Thermal decomposition of nitrocellulose to produce nitrites followed by migration to the drug product. ❖ Eg: Nitrocellulose coated blister foils. ❖ Biodegradation of nitrocellulose to produce nitrites followed by migration to the drug product.

1.2.1 Potential Source of N-nitrosamine Impurities in Drug Substances and Drug Products:



A. Staring materials, KSM Intermediates

Degradation processes of starting materials and intermediates induced by inherent reactivity (e.g. presence of nitro, oxime, or other functionality) or by the presence of an exogenous nitrosating agent.

- Sodium nitrite (NaNO2) or other nitrosating agents in the presence of secondary, tertiary amines or quaternary ammonium salts within the same or different synthesis step in which these compounds are carried over due to incomplete depletion.

- Sodium nitrite (NaNO2) or other nitrosating agents in combination with reagents, solvents and catalysts, from which secondary or tertiary amines are generated by degradation reactions.

- Use of contaminated starting materials and intermediates supplied by vendors who use processes or raw materials which may contain residual nitrosamines or nitrosating agents allow nitrosamine formation.

- Carryover of nitrosamines deliberately generated (e.g. as intermediates) during the manufacturing process.

- The evaluation should take in consideration number and type of chemical steps between introduction of raw material and final API stages.

- If the raw material is used in early or late steps of the API synthesis and no further crystallization is performed.

- If the next step(s) can purge the impurities or not, and if yes at which level the impurity can be purged.

Type of chemical process (es) is involved and process conditions.

Example:

- The catalyst Tri-N-butyl tin chloride (used as a source of tri-n-butyl tin azide) was contaminated at a third-party contractor facility due to the combination of this catalyst from different customers.

- There is a risk of nitrosamine formation when a quenching step is performed directly in the main reaction mixture (i.e., when nitrous acid is added to the reaction mixture to decompose residual azide). This allows nitrous acid to come into direct contact with residual amines in the raw materials used in the manufacturing process.

Presence of nitrosating agent and Nitrosatable substance or by-products.

Sr. No	Nitrosating Agent	N-Nitrosatable Substances (N-nitrosatable substances are nitrosamine precursors)	
		Nitrosatable substance	By-products
1	Nitrite Salt (MNO_2)	Cyclic and acyclic secondary amines	--
2	Nitrate Salt (MNO_3)	Tertiary amines	NHR_1R_2, NHR_1R_1, NHR_1R_3, NHR_2R_2, NHR_3R_3
3	Nitrous acid (HNO_2)	Hydrazine Derivatives	NHR_1R_2
4	Nitrous acidium ion ($H_2O^+ NO^-$)	N-methyl-2-pyrrolidinone	N-methyl-4aminobutricacid
5	Nitric acid (HNO_3, contains N_2O_4)	Tertiary amides	NHR_2R_3
6	Alkyl Nitrate R-ONO	N-Chloroalkyl amines	NHR_1R_2
7	Peroxynitrite NO+	N-alkyl carbamates	NHR_2R_3

B. Solvents

Amide solvents (e.g. DMF, DMAc and NMP): which are susceptible to degradation under certain reaction conditions, are another source of secondary amines.

Example:

- Under high reaction temperatures N, N-Dimethylformamide can degrade into dimethylamine, which can react with nitrous acid to form NDMA.

- N-Methylpyrrolidone and N, N-dimethylacetamide also have similar degradation pathways to form secondary amines that can react with nitrous acid to form nitrosamine impurities.

- Secondary amines could also be present as impurities in amide solvents. Example, dimethylamine, which can react with nitrous acid to form NDMA, may exist as an impurity in N, N-Dimethylformamide.

Recovered solvent:

- Recovered solvents may pose a risk of nitrosamine impurities due to the presence of residual amines (such as trimethylamine or diisopropylethylamine).

- If the recovery process involves a quenching step (i.e. nitrous acid used to decompose residual azide), nitrosamines could form during solvent recovery.

- Recovery and redistillation of solvent is often outsourced to third-party contractors. This outsourcing can pose a risk if the third-party recovery facility does not receive enough specific information on the contents of the materials they are processing and relies solely on routine recovery processes.

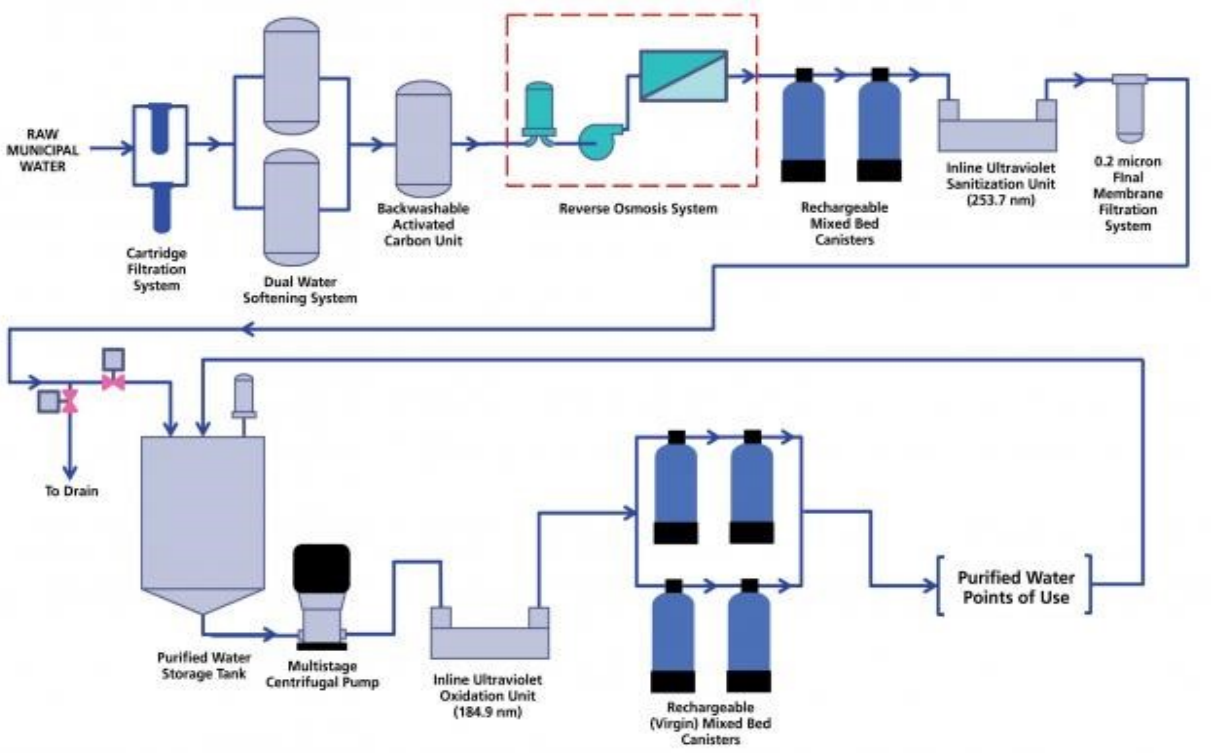
A manufacturing site may produce the same API by more than one synthetic process that uses common solvents. If any of those synthetic processes produces nitrosamines or contains precursor amines, the solvents sent for recovery are at risk. The use of recovered solvents that are comingled from different processes or across manufacturing lines without control and monitoring can introduce nitrosamine impurities.

C. Water

- NDMA can be expected to form during chlorination of surface waters containing ammonia due to the inevitable formation of monochloramine.

- Ozonation of drinking water contaminated with the fungicide tolyfluanide can also lead to the formation of NDMA.

Typical flow of Purified water system as below:



- Well water and tap water can contain nitrites and nitrates. Nitrite content is limited on a country by country basis. In EU the limits are typically between 0.1 and 0.5 ppm for well-water and tap water, as other global countries. If inhouse treatment (e.g. addition of chloramines) is used the impact on the level of nitrosamines formation should be assessed. For APIs manufactured in countries outside Europe, specific analysis of the nitrite or nitrate content in water might be considered.

- It is generally known that the level of nitrite in purified water is typically low, therefore purified water is considered as a low risk. In particular, steps of water carbon treatment are known to lower the nitrosamine content. On the contrary, ozonation of the water (containing traces of secondary amines) can theoretically lead to formation of nitrosamines.

- In several places, nitrile content may not be controlled in incoming water supplied, therefore in such cases nitrite or Nitrosamine presence (if amines involved) should be assessed, considering that in general no nitrite limit is established for tap water.

- Presence of Nitrosamines in potable water, Nitrosamine like NDMA is known to be present in ground water.
    - It could be by product in industrial processes.
    - Contaminant in pesticides.
    - Formed as a by-product of anion exchange treatment of water.
    - Presence of nitrosating agents in water such as
        - Presence of nitrites
        - Presence of chloramine

D. Cross Contamination and cleaning of Process Equipment

- Cross-contamination can take place due to different processes being run successively on the same manufacturing line. Carryover of impurities between process steps due to operator related errors or insufficiently detailed batch records such as inadequate phase separations during workup procedures.
- In case of multipurpose equipment, the potential carryover (nitrites, nitrosating agents, amines, etc.) should be assessed and if there is a risk, it should be mitigated by setting appropriate cleaning limits (e.g. by a corresponding control strategy).
- Some cleaning solvents (such as dimethylformamide) should also be expressly considered in the risk assessment.
- A general API have below important manufacturing steps;
- Dissolution, Mixing, reaction & unit operation, Filtration, Centrifugation, drying, milling, micronizing & shifting operation. For every operation specific design of equipment used based on the nature of process and unit operations.
- Below are the API manufacturing equipment's

Reactors

Sparkler Filter

Agitated Nutch Filter

Centrifuge

Rotacone Vacuum dryer

Fluid Bed dryer

Vacuum Tray Dryer

Multimill

Micronizer

Sifter

Below are the Drug Product manufacturing equipment's

Compression machine

Coating machine

Rotary Blender

Fluid bed dryer

Tray Dryer

Filling Machine

Automatic Capsule Filling machine

Automatic blister packing machine

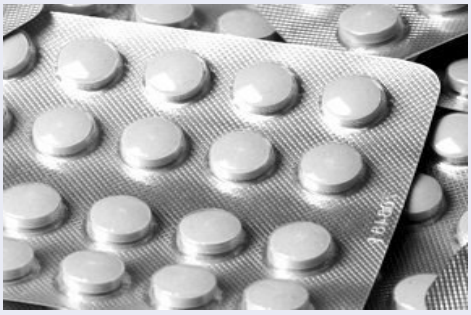
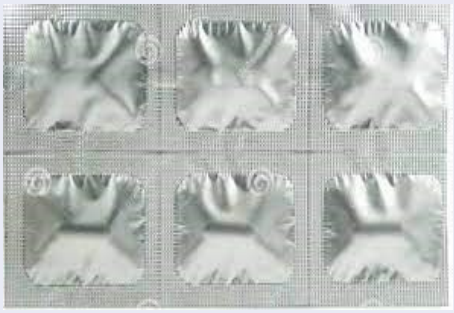
E. Presence of Nitrosamines due to shared facility may be at risk or no risk products.

- API/DP manufactured in multipurpose equipment exposed to nitrosating agents
- API/DP manufactured in multipurpose equipment > dedicated equipment.
- API > Intermediate > KSM (for companies manufacturing the three agents)
- API/DP manufactured in multipurpose equipment > dedicated equipment.
- API > Intermediate > KSM (for companies manufacturing the three categories).

Steam, cleaning agents other than solvents, and consumables (gaskets) should also be considered as applicable, depending on the chemical processes involved.

F. Packaging Materials

- Nitrocellulose is used as a polymer in the binding agent of solvent based inks. Nitrites are formed due to the breakdown of nitrocellulose compounds at higher temperature, which have greater tendency to migrate through packaging. These nitrite ions may react with the amines present in the drugs or printing inks itself to form carcinogenic nitrosamines. The concentration of nitrosamines tends to increase over time, and their formation is enhanced by high temperatures.
- Primary packaging materials, such as blisters in which the nitrocellulose cover film reacts with amines in the paint primer, generating nitrosamines, which would be transferred to the product during the packaging process.
- Elastomers used in packages for injectable and inhalable medicinal products. During compounding of rubber, secondary amines are likely formed from vulcanization accelerators.
- Presence of Nitrosamines in the Packaging.
    - Presence of nitrosatable Amines in the printing Ink, paint primer used in the printed primary packaging materials – Blisters.
    - Presence of Nitrosating Agents such as nitrocellulose in the printing primer used during printing process of the packaging materials – Blisters.
- In packages for injectable and inhalable medicinal products:
    - Presence of secondary amines i.e. formation of same from vulcanization accelerators. Since, Elastomers used during compounding of rubber.

Blister Pack		Strip Pack	
Ampules		Vials	
Bottles		Sachet pack	

Primary packaging: The most used material is polyethylene. Particular attention should be paid to the ones with additives such as antistatic packaging (which might contain specific additives) or packaging for liquid APIs by assessing potential interactions between packaging and API.

HDPE container		Fibre board drum	
LDPE bags		Triple laminated bags	

G. Nitrogen

Risk assessment should be considered because of the presence of NOx (nitric oxides) which might be considered as nitrosating agents, alternatively the demonstration of a proper control of nitrogen to prevent the presence of NOx can be relevant.

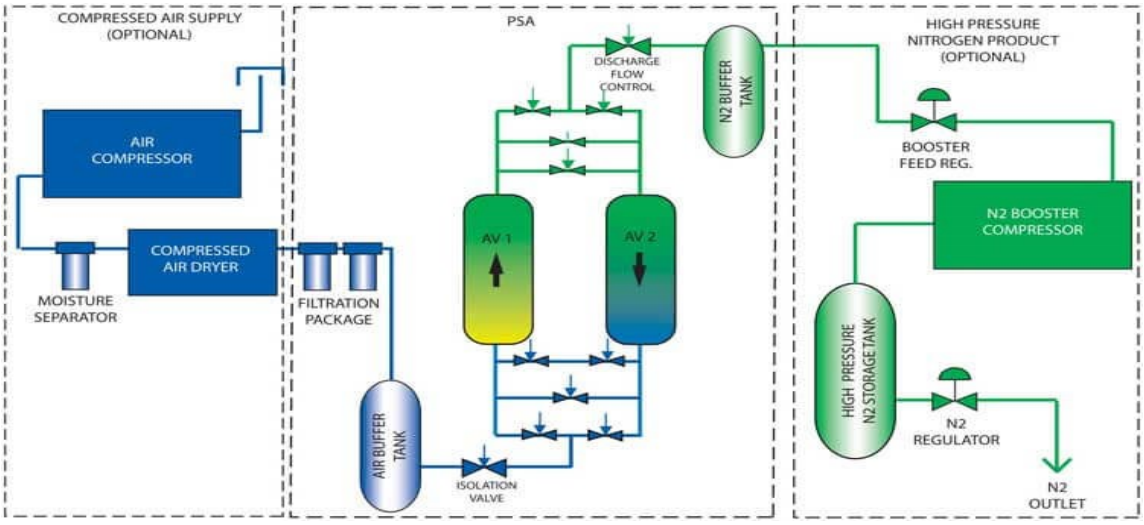


Diagram of Nitrogen generation system

H. Excipients

- Nitrosamine impurity can be generated during the manufacturing process of excipient or due to usage of input materials i.e. reagents, catalyst, raw materials or due to presence of nitro sating agents in the excipients.

- Precursors of reagents/catalysts/processing aids used in the manufacturing process.

- Water used for manufacturing of the excipients.

- Secondary and/or tertiary amine source as Raw material, Intermediate, Reagent, Processing aids, Catalyst / Base, Solvent.

- Amide, primary amine or ammonium salt used or present in the excipient manufacturing process as Intermediate, Reagent, Processing aids, Catalyst / Base, Solvent, Washing fluid.

- Use of recovered / recycled solvents in excipient manufacturing process.

- Use of multipurpose facility amongst different excipients manufacturing at site.

I. Gasket

- Elastomers used during compounding of rubber may be sources of the secondary amine formation. Leachable Nitrosatable agents may react with nitrosating agents available during the Finished drug product (FDP) manufacturing process and other components of FDP to form nitrosamines in FDP.

- The secondary amines present in the rubber may get converted into respective nitrosamines and carry forward into FDP.

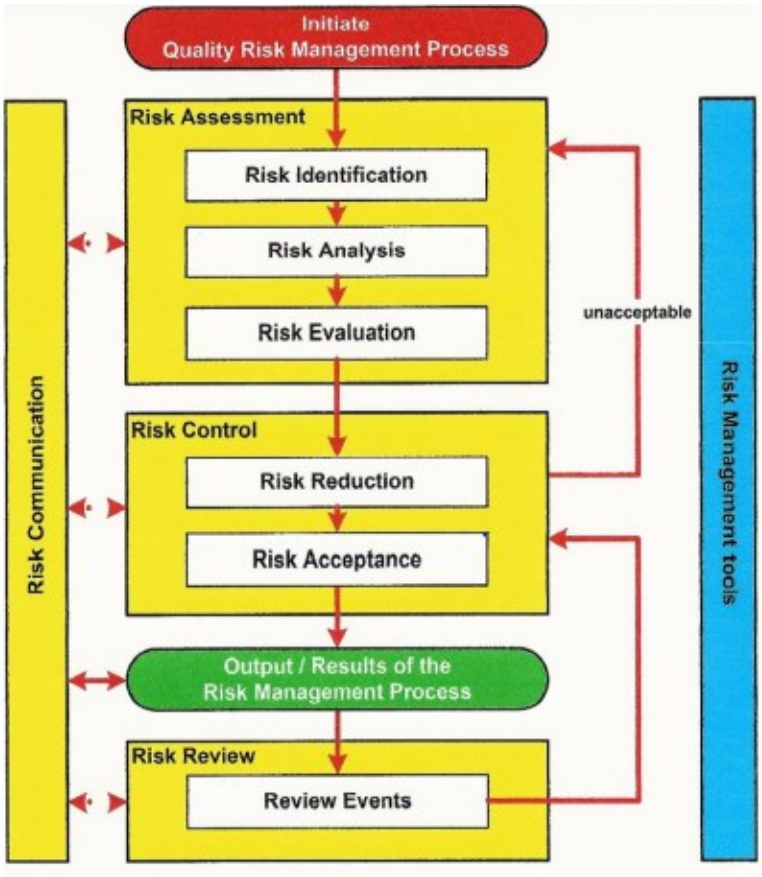
2 Risk Assessment / Quality Risk Management:

Two primary principles of quality risk management are:

- The evaluation of the risk to quality should be based on scientific knowledge and ultimately link to the protection of the patient; and

- The level of effort, formality and documentation of the quality risk management process should be commensurate with the level of risk.

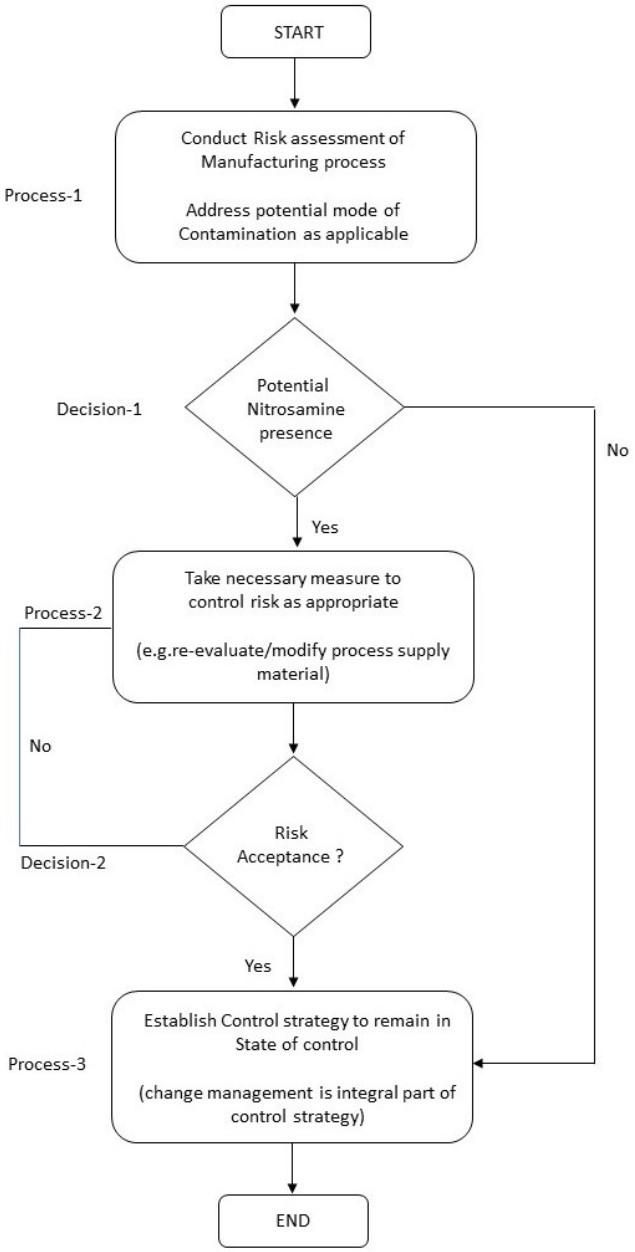
Quality risk management is a systematic process for the assessment, control, communication and review of risks to the quality of the drug (medicinal) product across the product lifecycle. A model for quality risk management is outlined in the diagram below. Other models could be used. The emphasis on each component of the framework might differ from case to case but a robust process will incorporate consideration of all the elements at a level of detail that is commensurate with the specific risk.



Overview of a typical quality risk management process

- Decision nodes are not shown in the diagram above because decisions can occur at any point in the process. These decisions might be to return to the previous step and seek further information, to adjust the risk models or even to terminate the risk management process based upon information that supports such a decision. Note: “unacceptable” in the flowchart does not only refer to statutory, legislative or regulatory requirements, but also to the need to revisit the risk assessment process.

- Although one of the sources with the highest potential for nitrosamines is the drug substance synthetic route, the drug substance manufacturing process, drug product manufacturing process, and excipients and raw materials should also be included in a risk assessment to establish if controls or additional controls are needed. An example of high-level process flow for evaluating materials is as below:



High level process for development of a nitrosamine impurity control strategy.

- In all the cases, if nitrosamines are predicted by the risk assessment or confirmed to be present through testing of the drug substance, drug product, or other materials, a control strategy should be defined an approach to ensure that the nitrosamine levels comply with the established Acceptable Intake (AIs).

- API manufacturers should review their manufacturing processes and perform risk assessments to identify the potential for nitrosamine impurities.

- If a risk of nitrosamine impurities is identified, confirmatory testing of batches should be conducted using sensitive and appropriately validated methods.

- If the risk assessment determines that there is no potential for nitrosamine impurities, there is no need to take further action.

- If a nitrosamine impurity is detected, API manufacturers should investigate the root cause. They should implement changes in the manufacturing process to reduce or prevent nitrosamine impurities. The changes recommended should be evaluated with appropriate Stability studies and regulatory updates.

- Risk assessments should be conducted in a timely manner based on the prioritization of drugs.

- Manufacturers do not need to submit risk assessment documents to the Agency, but they should retain these documents so that they are available if requested.

- The control strategy should be aligned with the current regulatory requirements.

- Risk assessment shall be performed as below;

2.1 Prioritization of the Risk Assessment

The following criteria might be used for prioritization (“>” meaning “higher priority than”) depending on the information available:

- Higher daily dose taken.
- Long duration of treatment.
- Therapeutic indication.
- Higher number of patients treated.
- Commercial APIs > APIs used for clinical trials.
- API manufactured in multipurpose equipment > dedicated equipment.
- API manufactured in multipurpose equipment exposed to nitrosating agents.

- API > Intermediate > KSM (for companies manufacturing the three categories).
- APIs still manufactured > APIs no longer manufactured but still on the market.
- APIs sold to markets where risk assessment have already been requested by authorities > APIs sold to other markets.
- Knowledge of the likelihood of a risk based on the chemistry of the process (presence of amine, nitro functionalities, nitrosating agents).

2.2 Management of raw materials in the risk assessment

The need and type of information to be obtained from suppliers depends on the type of material and on its use in the manufacturing process. Following factors are helpful to assess the impact of the raw materials on the risk to have nitrosamines in the API

2.2.1 Chemistry of the raw material manufacturing process, such as:

- Complex process.
- Use of nitrosating agents and amines.
- Type of solvents used (e.g. recovered in house or by 3rd party).

2.2.2 Number and type of chemical steps, between introduction of raw material and final API stages. The evaluation should take in consideration:

- If the raw material is used in early or late steps of the API synthesis.
- If the next step(s) can purge the impurities or not, and if yes at which level the impurity can be purged.
- Type of chemical process(es) is involved and process conditions.
- If the next step(s) can purge the impurities or not and if yes at which level.
- If the raw material is used in later stages of the API synthesis and no further crystallization is performed.

2.2.3 The information from raw material suppliers can be also obtained through questionnaires, which cover chemical process and risk of contamination at the raw material supplier's facility.

- Does supplier manufacture products associated with nitrosamines, amines or sources of amines in the same equipment as those used to manufacture the raw material?

- Does supplier use or is there a potential presence of nitrosating agents and/or amines (secondary or tertiary amines) in the manufacturing process?

2.3 Risk assessment approach

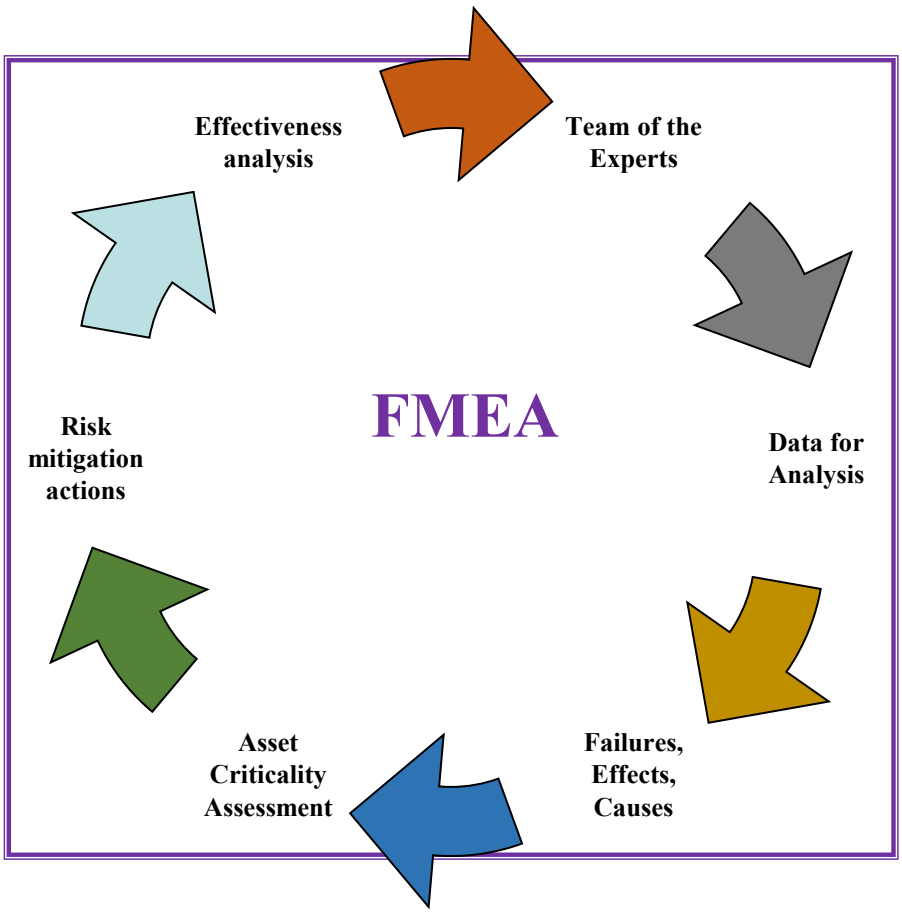
2.3.1 To identify the potential source of Nitrosamine impurities that may find their way into Drug product.

2.3.2 Below are the key elements and major contributors which could be the risk factor of nitrosamine impurity generation and carryover.

- Drug Substance.
- Utilities.
- Excipients.
- Manufacturing Process.
- Container Closure.
- Storage & Stability.

2.3.3 Methodology and outcome of the risk assessment

- he risk assessment can be performed through a suitable risk assessment tool as per ICH Q9 (Titled" Quality Risk Management) guideline i.e.
    - Failure Mode Effects Analysis (FMEA).
    - Failure Mode, Effects and Criticality Analysis (FMECA).
    - Fault Tree Analysis (FTA).
    - Hazard Analysis and Critical Control Points (HACCP).
    - Hazard Operability Analysis (HAZOP).
    - Preliminary Hazard Analysis (PHA).
- FMEA type tool with different scores assigned to various risk levels, through a yes/no questionnaire which has the benefit of better orienting the result. An example of a questionnaire is attached for reference as annexure.



- The outcome of the risk assessment can be, a high/medium/low risk to further establish planning and priority of next steps (in particular analytical testing), so that the API producer can prioritize further mitigation measures

- The decision to proceed to analytical testing should be taken, only when a risk is confirmed and the associated testing strategy can be unambiguously established. An ICH M7 (Titled: Mutagenic impurities) assessment is also a useful step to be undertaken before proceeding to any analytical testing.

- As the risk assessment has to address routine and accidental presence of nitrosamines in APIs, the definition of the boundaries of the risk assessment should be justified. A difference should be made between "possibility" to have nitrosamine and "likelihood" to have nitrosamines.

- Risk assessments shall be carried out considering following parameters, this is typical checklist however manufacture can be extend based on the knowledge and requirements.

2.3.4 Risk assessment checklist:

Risk assessment shall be carried out considering below check points

- Whether the Sodium Nitrite, Nitrate (NO3-) or other Nitrosating agents potentially present in the input materials (any raw materials, solvents, reagents, catalysts, water and steam, auxiliary materials such as filter materials, gaskets, and silica gel or work-up reagents) and intermediates? (Based on the review of the Route of synthesis), If yes, provide the details in which input material Nitrite or Nitrate can be present.

- Review of route of synthesis.

- List of Key Starting Material used in the process.

- List of Solid Raw materials used in the process.

- List of Solvents used in the process.

- List of Cleaning Solvents/Agents, Reagents (Specify the Name), Catalysts (Specify the Name)

- Water (Process/Purified etc.), Is chloramine formed as a byproduct in your water treatment and as a consequence can chloramine be present in the water used for cleaning or as part of the production process?
    - Note: chloramine is known to promote degradation of some relevant API structures to NDMA

- Is potable water (recycled or not) used in the manufacturing process where any nitrosating agents are used?

- If recycled water is used, comment on the potential for cross-contamination

- Usage of uncleaned equipment.

- Primary Packaging Material, Usage of Printing ink (for foil and Labels)

- Stability and Storage

- Usage of any Secondary or Tertiary amines in the manufacturing process?

- Possibility of product degradation into Secondary/Tertiary amine.

- Whether the usage of Sodium Nitrite / any other Nitrites used in presence of secondary and Tertiary amines at the same step or in previous steps?

- Are use recycled solvents, reagents and catalysts in the manufacturing process?
    - If so, how are they controlled and monitored within the waste streams sent for recovery, for the presence of Nitrosating agents and amines:

- The recycling process of solvents, reagents and catalyst does involve the quenching step with Nitrous acid?
    - If yes, the specification of such recycled solvents, reagents and catalysts does include the test for Nitrosamines?
    - Note: Examples of recycled materials observed to be contaminated with nitrosamines include Orthoxylene and tributyltin chloride (used as a source of tributyltin azide). It has also been suggested that N, N-Dimethylformamide (DMF) could be contaminated in this way.

- The recycling process of solvents, reagents and catalysts include the aqueous washes or distillations i.e. principles of boiling point / solubility properties
    - If yes, whether the boiling points or solubility properties of the solvents, reagents and catalysts under recycling, are same with that of Nitrosamine impurities?
    - If so, whether the specification of recycled solvents, reagents and catalyst include the test for Nitrosamine content?

- Does your manufacturing process involve the usage of recovered solvents / reagents/ catalysts?
    - If yes, the recovery process is performed inhouse or is outsourced?

- If the recovery process performed in-house, whether the equipment is dedicated to process the recovered solvents only for one product or multiple products?

- If it is for multiple products, whether the manufacturing process of other products for which, the solvents are recovered with common equipment, all the other products are evaluated for Nitrosamine impurities through the steps detailed above.
    - Note: This check shall be applicable even the recovery process is outsourced as stated above.

- Whether the third party used for recovery of solvents, catalysts and reagents is qualified through the evaluation process w.r.t. potential possibility of formation and control over the Nitrosamine impurity formation.
    - If Yes, elaborate the evaluation process and established criteria:
    - If No, the third party shall be qualified through the onsite audit considering the checks detailed above.

- The evaluation of the third-party recovery process, include the review of line clearance/ change over process between handling of the products from different customers?

- Whether the product (API / Excipients) upon degradation yields nitrosation reagent / dialkylamines?
    - If yes, please specify the impact of these degradation products on formation of nitrosamine impurities.
    - List all nitrosamine impurities identified from above risk assessment, in tabular form with actual compound names and structural formulae.

- Risk mitigation plan shall be summarised, if a nitrosamine risk is identified based on the risk evaluation.
    - Note: Refer the attached template to conduct the risk assessment for KSM & Intermediate, API and FDF. Annexure-I to IV.

2.3.3 Lifecycle of the risk assessment

The risk assessment is a live document which will be updated whenever additional knowledge is obtained on the API or process change is conducted (when risk assessment may need repeated). Mitigation actions should be defined if a risk is identified. If new information is obtained, such as late supplier information, and such information increases the risk level versus the previous version of the risk assessment, such new information will have to be communicated to the customers accordingly. The results of analytical testing change control and investigation systems should also feed the risk assessment. Impact on existing risk assessment shall be evaluated in the case of followings;

- ❖ Change in process.
- ❖ Change in Source water.
- ❖ Change in ROS of vendor.
- ❖ Change in vendor.
- ❖ Change in specification.
- ❖ Any updates from regulatory/Supplier.
- ❖ Pharmacopeial updates if any.

Mitigating the presence of Nitrosamines:

The following Mitigation strategies can help control/eliminate the formation of nitrosamines:

a) Optimize the design of the manufacturing process for APIs during route of synthesis (ROS) development to minimize or prevent the formation of nitrosamine impurities.

E.g.: avoid use of nitrites/nitrosatable substances/reaction conditions that promote the formation of nitrosamines (Nitrosamines are typically known to be formed during acidic conditions).

b) Use appropriate purge studies to demonstrate that the process is sufficiently robust to remove nitrosamines from the reaction.

c) Replacing nitrites with other quenching agents for azide decomposition processes.

d) Optimize the sequences of reactions, processes, and reaction conditions (such as pH, temperature, and reaction time).

E.g.: Consider removing or reducing quenching steps (when there is a risk of nitrosamine formation, e.g., using nitrous acid to decompose residual azide) from the main reaction mixture to reduce the risk of nitrosamine formation.

e) Commonly used antioxidants such as ascorbic acid (Vitamin C) or alpha-tocopherol (Vitamin inhibit the formation of nitrosamines

f) Audit supply chains and monitor them for any at risk raw materials, starting materials, and intermediates.

g) To avoid cross-contamination when recovered materials such as solvents, reagents, and catalysts are used in the manufacturing process, recovered material should be used only in the same step or in an earlier step (if there is sufficient purification) of the same process from which it was collected.

h) If the recovery of materials is outsourced to third party contractors, the API manufacturer should audit the facility for its adequacy of validation of cleaning procedures.

i) Potable water used in API manufacture may contain low levels of nitrite and even nitrosamines from environmental contamination. Therefore, analyse nitrite and nitrosamine levels in water and use water that has been purified to remove unacceptable impurities.

2.3.3 Analytical Testing;

The outcome of the risk assessment performed for the API manufacturing process will determine the need for analytical testing, when there is any risk for the presence of nitrosamine impurities. Due to nitrosamine's physiochemical properties (low molecular weights, some volatility and high toxicity), the analytical methods for nitrosamines need to have specificity, excellent chromatographic separation, and highly sensitive detection capability. Below table provides the recommendations from various regulatory agencies from

Regulatory	Omission	Skip testing	Routine control
EMA	the LoQ of the analytical method employed should be ≤ 10% of the acceptable limit based on the AI	the LoQ of the analytical procedure employed should be ≤ 30% of the acceptable limit based on the AI	the LoQ should be ≤ of the acceptable limit based on the relevant acceptable intake (AI) for the respective nitrosamine impurity
FDA	Alternate approaches (e.g., upstream test of an intermediate) should be supported by sufficient process understanding and evidence of adequate statistical control and should be submitted to FDA in a supplement prior to implementation		If a nitrosamine impurity is detected above the LOQ
ANVISA	Admitted the absence of nitrosamines when <10% of the AI limit	If results are >10% of AI limit, control must be included. Other approaches can be justified, not exceeding the 30% limit. If the >1 nitrosamine to be controlled, the limits must be adjusted in order to ensure the maintenance of negligible risk	
SWISSMEDIC	The detection of every nitrosamine impurity must lead to an investigation of the causes, and appropriate CAPAs should be taken in accordance with GMP. As with any case of an identified problematic risk, companies must follow the standard procedure and inform Swiss medic immediately if nitrosamines are detected in APIs or medicinal products – regardless of the quantities – and submit a risk evaluation.		
HEALTH CANADA	Analytical procedures may need to be validated with LOQs well below the most conservative AI limit of the nitrosamines present, if proposals for a reduced testing program or absence of testing of the drug product are anticipated.		The API specification should include a test and acceptance criterion for each nitrosamine impurity when the risk for nitrosamine presence is considered to be high and/or when the concentration of any nitrosamine is found to be at significant levels (e.g. greater than 30% of the acceptable intake) during confirmatory testing.

3 Control Strategy

In order to determine the level of control, if any, which requires ensuring that levels of nitrosamines are at or below the provisional acceptable intake (AI) if their presence could not be avoided, the components of drug products should be assessed for the potential to form nitrosamines or to be contaminated with nitrosamines. Although one of the sources with the highest potential for nitrosamines is the API synthetic route, the API manufacturing process, drug product manufacturing process, and excipients and raw materials should also be subjected to a risk assessment to establish the level of control needed.

3.1 Control strategy of 'Identified Potential Nitrosamines' in cleaning samples:

- Cross-contamination can take place due to different processes being run successively on the same manufacturing line.

- In case of multipurpose equipment, the potential carryover (nitrites, nitrosating agents, amines, etc.) should be assessed and if there is a risk, it should be mitigated by setting appropriate cleaning limits (e.g. by a corresponding control strategy).

- Some cleaning solvents (such as dimethylformamide) should also be expressly considered in the risk assessment.

- Also, the other risk factor for API is Carry-over of nitrosamines from the previously manufactured product (which is identified with potential nitrosamine impurities) to subsequent product manufacturing.

- Hence, it is essential to perform cleaning validation for the products with potential of Nitrosamine impurities manufactured in shared facility irrespective of the level.

- It is recommended to define and prepare a control strategy for the cleaning samples analysis for the risk identified APIs and other different processes being run on the same manufacturing line.

- As a general recommendation, at least three batch equipment cleaning cycles to be carried for the testing of identified Nitrosamine impurities content using a suitable sensitive and validated method.

- Control strategy shall be finalised based on the outcome of assessment.

3.2 Control Strategy Lifecycle

- As per ICH Q10 (Titled: Pharmaceutical Quality System) and the summary in ICH M7, a set of controls based on process understanding and risk management principles (ICH Q9 – Quality Risk Management) should be defined to assure process performance and product quality is defined as Control Strategy.

- From the risk assessment and the evaluation of the level of the nitrosamine(s) impurity(ies) a specific testing frequency or any other control should be defined to assure that the level of the impurity(ies) will be kept under control and below of the acceptance limit, across the product lifecycle.

- The four (4) possible control options described in ICH M7 should be evaluated based on the process understanding, the impurity(ies) level and type and manufacturing step where it is formed.

- The option chosen should be fully justified based on scientific principles, analytical data, the knowledge on the downstream process and impact on the impurity level.

- The control strategy effectiveness and process performance should be assessed periodically. The knowledge gained from the commercial manufacturing should be used to encourage the continuous improvement and adjustment of the control strategy. Manufacturing continuous improvement may include manufacturing process changes.

- Any proposed process changes independently of the type of change (raw Qmaterials, suppliers, analytical methods, manufacturing step, etc.) and based on the understanding of the manufacturing process, should include the impact assessment on several areas such as, but not limited to:

    a. Impurities level and the possibility of new impurities be formed either due to side reactions or due to new solvents, reagents, water, etc. In the case of new impurities being formed, ICH M7 guideline should be followed.

    b. Cleaning process: If it is still valid or needs to be adjusted, including composition assessment of cleaning agents.

    c. New solvent/reagent/catalyst used and respective supplier qualification with focus on the product origin (recovered or not).

    d. Internal use of recovered materials, the result of the impact assessment exercise may originate adjustments in the control strategy to assure process performance and product quality. All changes should be handled through the change management process in place as part of the organization quality management system.

3.3 Setting Limits:

- Nitrosamine impurities identified have potential and established toxicity with no therapeutic value. Because nitrosamines are among the structural groups of high potency mutagenic carcinogens of the “cohort of concern” in ICH M7, the threshold of toxicological concern (TTC) does not apply

- Instead, the available safety data should be used to establish a material specific AI on case by case basis.

- The AI is defined as an intake level that poses a negligible health risk.

3.4 Derivation of AI Limits:

- There are several methodologies that toxicologists have applied in establishing. The below limits have been published in the FDA Guidance for Industry to Control of Nitrosamine Impurities in Human Drugs. A description of the AI derivation for NDMA, which is an example of how FDA applied ICH M7(R1) to set a limit.

- The conversion of AI limit into ppm varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug label (ppm = AI (ng/day)/MDD (mg/day).

3.5 Acceptable Intake Limits:

- The following limits have been established for some specific N-nitrosamines and we further recommend that manufacturers use these AIs when determining limits for nitrosamine impurities in APIs and drug products.

Impurity	Code	CAS No.	EMA	FDA	ANVISA	Swiss Medic	Health Canada
			(ng/day)	(ng/day)	(ng/day)	(ng/day)	(ng/day)
N-Nitrosodimethylamine	NDMA	62-75-9	96.0	96.0	96.0	96.0	96.0
N-Nitroso-4-(methylamino)butyric acid	NMBA	61445-55-4	96.0	96.0	96.0	96.0	96.0
1-Methyl-4-nitrosopiperazine	MNP/MeNP	16339-07-4	26.5	-	26.5	26.5	96.0
N-Nitrosodiethylamine	NDEA	55-18-5	26.5	26.5	26.5	26.5	26.5
N-Nitrosodiisopropylamine	NDIPA/ DIPNA	601-77-4	26.5	26.5	26.5	26.5	26.5
N-Nitrosoethylisopropylamine	NEIPA/NIPEA/EIPNA	16339-04-1	26.5	26.5	26.5	26.5	26.5
N-Nitrosodibutylamine	NDBA	924-16-3	26.5	USP	26.5	26.5	26.5
N-Nitrosomethylphenylamine	NMPA	614-00-6	34.3	26.5	34.3	34.3	-
N-Nitrosomorpholine	NMOR	59-89-2	127.0	-	-	127.0	127.0
N-Nitrosovarenicline	NNV	-	37.0	-	-	37.0	37.0
N-Nitrosodipropylamine	NDPA	621-64-7	26.5	-	-	26.5	26.5
N-Nitrosodimethylamine	NDMA	62-75-9	96.0	-	-	N-Nitrosodimethylamine	NDMA
N-Nitroso-4-(methylamino)butyric acid	NMBA	61445-55-4	96.0	-	-	N-Nitroso-4-(methylamino)butyric acid	NMBA
1-Methyl-4-nitrosopiperazine	MNP/MeNP	16339-07-4	26.5	-	-	1-Methyl-4-nitrosopiperazine	MNP/MeNP

Impurity	Code	CAS No.	EMA	FDA	ANVISA	Swiss Medic	Health Canada
			(ng/day)	(ng/day)	(ng/day)	(ng/day)	(ng/day)
N-Nitrosodiethylamine	NDEA	55-18-5	26.5	-	-	N-Nitrosodiethylamine	NDEA
N-Nitrosodiisopropylamine	NDIPA/DIPNA	601-77-4	26.5	-	-	N-Nitrosodiisopropylamine	NDIPA/DIPNA
N-Nitrosoethylisopropylamine	NEIPA/NIPEA/EIPNA	16339-04-1	26.5	-	-	N-Nitrosoethylisopropylamine	NEIPA/NIPEA/EIPNA
N-Nitrosodibutylamine	NDBA	924-16-3	26.5	-	-	N-Nitrosodibutylamine	NDBA
N-Nitrosomethylphenylamine	NMPA	614-00-6	34.3	-	-	N-Nitrosomethylphenylamine	NMPA
N-Nitrosovarenicline	NNV	-	37.0	-	-	N-Nitrosovarenicline	NNV
N-Nitrosodipropylamine	NDPA	621-64-7	26.5	-	-	N-Nitrosodipropylamine	NDPA
N-Nitrosomethylphenidate	-	-	-	1300	1300	N-nitrosomethylphenidate	-
N-Nitrosopiperidine	-	100-75-4	-	1300	1300	N-nitrosopiperidine	-
N-Nitrosorasagilene	-	-	-	18	-	N-nitrosorasagilene	-
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-a]pyrazine	-	-	-	37	37	7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine	-

Impurity	Code	CAS No.	EMA	FDA	ANVISA	Swiss Medic	Health Canada
			(ng/day)	(ng/day)	(ng/day)	(ng/day)	(ng/day)
N-Nitroso-1,2,3,6-tetrahydropyridine	-	55556-92-8	-	37	37	N-nitroso-1,2,3,6-tetrahydropyridine	-
N-Nitrosonortriptyline	-	-	-	8	8	N-nitrosonortriptyline	-
N-Methyl-N-nitrosophenethylamine,	NMPEA	13256-11-6	-	8	8	N-methyl-N-nitrosophenethylamine,	NMPEA
N-Nitrosodabigatran	-	-	-	18	18	N-Nitrosodabigatran	-
N-Nitroso-duloxetine	NDLX	-	-	-	100	N-nitroso-duloxetine	NDLX
4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone)	NNK	-	-	-	100	4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone)	NNK
N-Nitroso-rasagiline	-	-	-	-	18	N-nitroso-rasagiline	-
N-Nitroso-tamsulosin	-	-	-	-	18	N-nitroso-tamsulosin	-

EMA Reference:

- These limits are applicable only if a drug product contain a single nitrosamine.
- If Nitrosamines are identified without sufficient substance specific data to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1) guideline,
- A classic specific TCC for nitrosamines of 18 ng/day (derived from the Lhasa carcinogenic potency database) can be used as default option.

USFDA Reference:

- If more than one of the nitrosamine impurities (NDMA, NDEA, NMBA, NMPA, NIPEA, NDIPA) is identified, detected and the total quantity of nitrosamine impurities exceeds 26.5 ng/day (the AI for the most potent nitrosamines) based on the maximum daily dose (MDD), the manufacturer should contact the Agency for evaluation.
- For drug products with an MDD of less than 880 mg/day, a recommended limit for total nitrosamines of 0.03 ppm is not more than 26.5 ng/day and is considered acceptable.
- For drug products with an MDD above 880 mg/day, the limit for total nitrosamines should be adjusted so as not to exceed the recommended limit of 26.5 ng/day.
- Note: Manufacturers should contact CDER if multiple nitrosamine impurities are detected in an API or drug product in which the total nitrosamine level exceeds 26.5 ng/day based on MDD.
- If nitrosamines without published AI limits are found in drug products, manufacturers should use the approach outlined in ICH M7 (R1) to determine the risk associated with the nitrosamine and contact the Agency about the acceptability of any proposed limit.
- Sensitive methods with limits of quantitation (LOQ) in the parts per billion (PPB) range are needed to meet the low AIs recommended for nitrosamines.
- Manufacturers of APIs and drug products should use methods with LOQs at or below 0.03 ppm.
- Manufacturers should establish methods for which the LOQ and limit of detection (LOD) are as low as reasonably practical for products for which the maximum daily dose is high (e.g., greater than 1 g).
- For example, if the MDD is 1200 mg, the LOQ should be below 0.02 ppm. FDA's public webpage includes validated analytical test methods recommended for detecting nitrosamine impurities in several different APIs and products.
- Note: The LOQ may be considered the reporting threshold for nitrosamine impurities (i.e. the limit above which an impurity should be reported in the certificate of analysis).

- If more than one nitrosamine listed in above is detected, then the below example of the different cases of impurities taking hypothetical example MDD 1000 mg/day.

- Example:

Sr. No.	Name of Impurities Identified.	Acceptable intake in ng/day	Maximum daily dose (MDD)	Acceptable Intake (AI) in ng/mg or ppm	Total impurities limit In ng/mg or ppm
1	NDMA	96	1000 mg/day	0.096	Not Applicable
2	NMBA	96		0.096	
3	NDEA	26.5		0.0265	
4	NIPEA	26.5		0.0265	
5	NDIPA	26.5		0.0265	
6	NMPA	26.5		0.0265	
7	NDBA	26.5		0.0265	
8	MeNP	26.5		0.0265	
9	NMOR	127		0.127	
10	NDMA NMBA	96 96	1000 mg/day	0.096 0.096	0.096
11	NDMA NDEA	96 26.5	1000 mg/day	0.096 0.0265	0.0265
12	New Impurity (TD50 available)	TD50	1000 mg/day	TD50/1000	Not applicable
13	New Impurity (TD50 not available)	18	1000 mg/day	0.018	Not applicable
14	NDMA New impurity (TD50 not available)	96 18	1000 mg/day	0.096 0.018	0.018
15	NDEA New impurity (TD50 not available)	26.5 18	1000 mg/day	0.0265 0.018	0.018
16	NDMA NDEA New Impurity (TD50 not available)	96 26.5 18	1000 mg/day	0.096 0.0265 0.018	0.018

4 Analytical Method Development:

- The following procedures have been established as suitable for their intended specified purpose. Users should validate these methods while considering the effect of sample solubility and extraction efficiency on the test results for other materials for which they are intended to be applied.

- Reference USP <1225> and Verification of Compendial Procedures <1226>.
  Following Quantitative procedures available in <1469> Nitrosamine Impurities.
  - HPLC-HRMS.
  - Headspace GC-MS.
  - HPLC-MS/MS.
  - GC-MS/MS (Triple-quad)

4.1 Nitrosamine impurities Analytical Method Development, Validation and regular testing:

- N-Nitrosamine, refer to any molecule containing the nitroso functional group. These molecules are of concern because nitrosamine impurities are probable human carcinogen. An article was discussed in detail about why Nitrosamine impurities are present, current available regulatory guidance, potential sources of Nitrosamines, testing method requirements, general recommendation from confirmatory testing and Life cycle management.

- Analysis of N-nitrosamines has been a hot topic in the pharmaceutical industry since 2018 when these potential carcinogens were found in several angiotensin II receptor blocker (sartans). Their subsequent discovery in Ranitidine and some slow-release Metformin prompted widespread product recalls.

- The sensitivity and reliability of analytical methods related to detection and quantification of nitrosamine impurities in pharmaceuticals is crucial due to the extremely low levels of these impurities determined acceptable by regulatory agencies. FDA has published several analytical methods that may be considered when determining nitrosamine content in active pharmaceutical ingredient (API) or finished pharmaceutical product (FPP). These methods include both liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) or high resolution mass spectrometry (HRMS). USP general chapter <1469> has published recommended quantitative analytical procedure and its performance criteria which was required during method development and validation.

Various Nitrosamines are considered for method development and validation

- N-Nitrosodimethylamine (NDMA)
- N-Nitrosodiethylamine (NDEA)
- N-Nitrosodiisopropylamine (NDIPA)
- N-Nitrosoethylisopropylamine (NEIPA)
- N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)
- N-Nitrosodibutylamine (NDBA)
- N-Nitrosoethylmethylamine (NMEA)
- Nitrosomethylphenylamine (NMPA)

4.2 USP published analytical methods:

Reference : USP <1469> Nitrosamine Impurities

Section: 8. Analytical Procedures

- Quantitative Procedures
  - Procedure 1: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA NMPA and NDBA in selected sartans (valsartan, irbesartan, and losartan potassium) by HPLC-HRMS
  - Procedure 2: Quantitation of NDMA, NDEA, NDIPA and NEIPA in selected sartans (valsartan, irbesartan, losartan potassium, Olmesartan medoxomil, candesartan cilexetil and telmisartan) by Headspace GC-MS.
  - Procedure 3: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans (valsartan, losartan potassium, Olmesartan medoxomil, candesartan cilexetil and telmisartan) by HPLC-MS/MS.
  - Procedure 4: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMPA and NDBA in selected sartan (valsartan, losartan potassium, and candesartan cilexetil) by GC-MS/MS (triple-Quad)

4.3 Key points to select/Choose Analytical Method for Nitrosamine impurities:

- For Nitrosamine impurities in Metformin, Valsartan, Losartan and Ranitidine drug substance and drug products, various methods have been published and tabulated below and can verify the methods to demonstrate the suitability of the test procedure for intended purpose.

Reference Analytical Methods for Nitrosamine impurities by LCMS/GCMS				
Sr. No.	**Products**	**Impurity Name**	**Analytical Technique**	**Reference**
1	Metformin drug product	NDMA	LCMS/MS with MRM	Sciex; RUO-MKT-03-12830-A
2	Metformin drug substance & drug product	NDMA, NDEA, NEIPA, NDIPA, NDPA, NMPA, NDBA, NMBA	LCMS (HRMS)	FDA method
3	Valsartan Drug substance	NDMA & NDEA	GC-MS-HS with SIM	FDA (FY19-005-DPA-S)
4	Valsartan Drug substance & Product	NDMA	GC-MS-HS with SIM	FDA method
5		NDMA, NDEA, NEIPA, NDIPA		FDA method
6		NDMA, NDEA, NEIPA, NDIPA, NDBA	GC-MS/Liquid Injection with MRM	FDA method
7		NDMA, NDEA, NDPA, NDBA, NPYR, NPIP, NMOR & NDELA	LCMS/MS with MRM	Sciex; RUO-MKT-02-9127-A
8	Losartan Potasium Drug substance & product	NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA, NMBA	LCMS (HRMS)	FDA method
9			LCMS/MS with MRM	FDA method
10	Ranitidine Drug substance & drug Product	NDMA	LCMS (HRMS)	FDA method

- For Nitrosamine impurities in other products apart from the above published methods, USP <1469> has published four procedures by various techniques GCMS/MS using Headspace and liquid injection, LCMS using high resolution and tandem mass spectroscopy, that procedures can verify and based on the suitability can adopt the method for intended purpose.

- If it requires to develop a new method need to check the below strategy of method development.

4.4 Strategy / approach for new method development:

Note: If both USP<1469> and USFDA published methods are not suitable for the product being analysed for the specified nitrosamine impurities, the following method development approaches are recommended.

LCMS: LC Optimization:

- Collect and review the literature for method related information on the molecule and use this for initial experiments, choose the default column as 150 x 4.6mm, 5µm or equivalent and select the bonding phase based on the polarity of the molecule and choose bonding phase with similar polarity as that of analytes. Try with mobile phases contains volatile buffers (Formic acid, Ammonium formate, Ammonium acetate and Ammonium bicarbonate, if required use trifluoroaceticacid based on application). Start the method development always using a gradient method. Initially start with a gradient of 50: 50 buffer and organic modifier and change the programme linearly up to 5: 95 and retain for at least 20 to 25 minutes.

- When mobile phase-A is only buffer and then sudden change in the gradient may causes for change in pressure cycle of over pressure. It could happen normally due to change in viscosity, then it is advisable to check pre-mix the mobile phase and run the gradient to control the pressure and understand the elution pattern of all the desired peaks, based on the elution with these gradient program further optimization shall be done.

- Choose a diluent in which impurities and API are soluble. It is advisable to check in mobile phase initially, all the analytes should be completely soluble and solution should be clear. Diluent should be compatible with the mobile phase to obtain the good peak shape. If required to verify the extraction procedure during sample preparation based on the application. Remove as many interferences as possible while maximising recovery of the analyte. Measure the effect of matrix on the ionization process and analyte recovery, adjust the LC conditions, if sufficient recovery is not achieved to separate out the interferences.

MS Optimization:

- Ensure the ionization of the analytes in the mass spectrometer and it is usually carried out by introducing a constant stream of analyte into the ion source using a syringe infusion pump and record a mass spectrum over a wide mass range. Tune the mass spectrometer parameter for desired analyte, such as gas flow and ionization voltage, to create the optimum conditions for ionization and therefore sensitivity. Choose the best ionization mode (positive/negative) based on the molecule and perform fragmentation study (MS/MS), then will get most informative fragments. Select a suitable precursor ion and product ions. Avoid selection of product ions associated with transitions that may prove

non-selective, i.e., loss of water. Optimize the nebulization process by ensuring the appropriate electrospray capillary voltages, nebulizer gas flow, sprayer position relative to the sampling orifice, eluent composition and flow rate. Optimize the desolvation process by keeping sufficient source temperate, gas flows and declustering potential. Optimize the collision energy, gas flow and associated voltages to ensure the degree of fragmentation of each precursor ion in the collision cell. Initially monitor two or three MRM transitions for each analyte, Co-elution of different components is acceptable as long as the mass can distinguish them and they do not interfere with ionization process. Evaluate the concentration of analyte to achieve the required signal to noise value since this will determine whether actual samples will need to be concentrated or diluted. Selectivity and sensitivity are affected by every stage of method development, based on the sensitivity and selectivity, SIM or MRM shall be used for analysis.

For GCMS: GC Optimization:

- Collect and review the literature for method related information on the molecule and use this for initial experiments, choose the default column as 30X0.25mm, 0.25µm or equivalent and select the stationary bonding phase (i.e., DB-Wax, DB-624 and DB-1) based on the polarity of the molecule. Start the method development using a gradient oven temperature programme and retain for at least 30 minutes. Column stationary phase, length, diameter and film thickness plays vital role in elution pattern, separation and peak shape. Choose inlet liner based on the sample concentration. If it is high sample concentration, select split liner and optimize the split ratio based on the sensitivity of the analyte and evaluate the flow rate to get the resolution and peak shape of analytes. Establish the Inlet temperature should be more than or equivalent to boiling point of the desired analyte and the column outlet connect to MS interface and the interface temperature should be higher than the column oven temperature.

MS Optimization:

- Ensure the ionization of the analytes in the mass spectrometer and it is usually carried out by introducing a constant stream of analyte into the ion source and record a mass spectrum over a wide mass range with default ion source temperature of 230°C. Tune the mass spectrometer parameters for desired analyte, such as detector gain mode, gain value and dwell time, to create the optimum conditions for sensitivity. Choose the best SIM ion from its mass spectrum which was generated in initial scan experiment and it should be stable and most abundant ion and it should not interfere with other impurities/solvents. Mass instrument tuning is utmost important to achieve sensitivity of desired analyte and it was depending on gas purity (always 99.999% is required), ion source cleaning and GC column fittings without any leakages.

Considerations for Sample Preparation

- Appropriate sample preparation is a critical step in trace impurity analyses such as those required to evaluate the levels of nitrosamines in drug substances and drug products. Special care shall be taken to prevent the loss or generation of nitrosamines as artifacts of the analytical procedure itself, as in the following circumstances.

- The presence of dialkyl amine (dimethylamine) as a process impurity or counter ion of the salt form of the active ingredient in the presence of nitrite and

- Acid can lead to in situ formation of nitrosamines as an artifact, especially in GC analyses.

- Total solubilisation versus selective extraction: If the active ingredient contains a dimethyl amino group, total dissolution of the drug substance should be avoided when applying GC techniques.

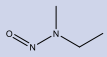
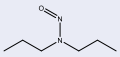
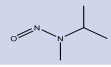
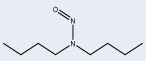
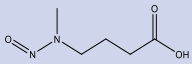
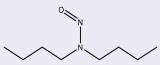
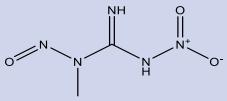
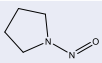
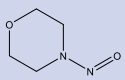
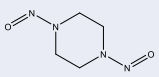
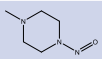
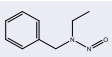
- High concentration of the active ingredient, when injected in the GC instrument can generate nitrosamines in the injection port if a nitrosating agent is present. In these situations, sample extractions should be modified to prevent the solubilisation of the active

- Ingredient while maintaining the extraction efficiency for nitrosamines present in the material

- Special emphasis should be placed on the workup procedures prior to injection of the sample solution in either GC or LC in order to reduce potential interferences. While older literature describes laborious liquid-liquid extractions followed by concentration steps (with the risk to lose volatile N-nitrosamines), combined extraction and concentration can be achieved with solid-phase extraction (SPE) or solid-phase micro extraction (SPME). Several materials can be used for SPE. Published review articles [Perera (2006); Boyd et al. (2011)] discuss the advantages and disadvantages of different materials, either used alone or in combination. Activated carbon adsorbents are considered as suitable in general while reversed phase materials are deemed less effective. However, a combination of both increases the recovery of N-nitrosamines. SPME extracts the volatile or semi-volatile analytes from solutions with fused-silica fibre coated with a polymeric liquid phase. After equilibration, the adsorbed or absorbed analyte on the fibre is thermally desorbed in a hot injector port of a gas chromatograph or in an appropriate interface of a liquid chromatograph [Perera (2006)].

- The hydrophilicity and consequently the solubility of the target analyte in water or other solvents should be taken into account, when elaborating the workup procedure. While NDMA and NDEA show a high-water solubility, the water solubility of NDPA and NDBA is lower. Some characteristics are given in table. Typical solvents used for extraction of N-nitrosamines are dichloromethane, methanol and acetone.

Table 5 : Chemical properties of Nitrosamine impurities

Chemical Name	Code	CAS	Structure	Pka	Water Solubility
N-Nitroso dimethyl amine	NDMA	62-75-9		-0.057	13.5 mol/L
N-Nitroso diethyl amine	NDEA	55-18-5		0.48	1.04 mol/L
N-Nitroso methyl ethyl amine	NMEA	10595-95-6		0.04	3.4 mol/L
N-Nitroso Dipropyl amine	NDPA	621-64-7		1.36	0.099 mol/L
N-Nitroso diisopropyl amine	NDIPA	601-77-4		1.38	0.099 mol/L
N-Nitroso ethyl isopropyl amine	NEIPA	16339-04-1		0.9	0.199 mol/L
N-Nitroso methyl isopropyl amine	NMIPA	NMIPA		-	-
N-Nitroso Dibutyl amine	NDBA	924-16-3		2.63	0.008 mol/L
N-Nitroso-N-methyl-4-amino butyric acid	NMBA	61445-55-4		-0.04	2.29 mol/L
N-Nitrosodiethanolamine	NDELA	1116-54-7		-1.29	7.45 mol/L
N-Nitroso methyl nitroguanidine (N'-Nitro-N-nitroso-N-methylguanidine)	NMNG / MNNG	70-25-7		-0.809	1.29 m0l/L
N-Nitroso pyrrolidine	NPyR	930-55-2		-0.19	9.99 mol/L
1-Nitrosopiperazine	-	5632-47-3		-	-
N-Nitroso piperidine	NPIP	100.75-4		0.36	0.67 mol/L
N-Nitroso morpholine	NNMP / NMOR	59-89-2		-0.44	8.61 mol/L
1,4-Dinitrosopiperazine	DNP	DNP		-	-
1-Methyl-4-nitrosopiperazine	MNP	MNP		-	-
N-Nitroso dicyclo hexylamine	NDCHA	NDCHA		-	-
N-methyl-N-Nitroso aniline	NMPhA	614-00-6		1.49	0.0494
N-Nitroso benzyl ethyl amine	NBEA	NBEA		-	-
N-Nitroso diphenyl amine	NDPhA	86-30-6		3.13	.000177 mol/L

❖ The following procedures have been established as suitable for their intended purpose. Use of these methods should be verified by the user for the specific materials for which they are intended to be applied. The objective of verification is to demonstrate the suitability of a test procedure under actual conditions of use (see Verification of Compendia Procedures 〈1226〉).

Typical Analytical Method by LC-MS for multiple Nitrosamine impurities quantification:

Column	Poroshell EC-C18 2.7 µm 4.6 X 150 mm						
Flow	0.5 ml/min						
Column temperature	30°C						
Needle Wash	Methanol: Water (650: 350 v/v)						
Mobile phase-A	1 mL formic Acid in 1000mL of Milli-Q Water, filtered through 0.22µ filter						
Mobile phase-B	Methanol						
Diluent	Water : Methanol (950 : 50, v/v)						
Gradient program	Time	0.01	3	15	19	20	25
	% A	99	99	10	10	99	99
	% B	1	1	90	90	1	1
Injection Volume	20µL						
Run time	25 minutes						
Mass Parameters							
Scan type	MRM						
Ion source	APCI						
Polarity	Positive						
CAD	Medium						
Temperature	450°C						
Curtain gas	30 psi						
GS1	55 psi						
NC	3						
EP	10						
CXP	9						
MRM Transition							
Impurity Name	Q1 MS	Q3 MS	Dwell Time	CE	DP		
NDMA	75.100	58.100	200	16	40		
NDEA	103.1	75.1	200	15	40		
NDIPA	131.1	89	200	15	40		
NEIPA	117.1	75	200	20	40		
NDCHA	211.1	129.0	200	15	50		
NDPA	131.1	89.1	200	20	40		
NNMP/NMOR	117.100	87.100	200	11	30		
NDBA	159.200	103.100	200	13	40		
NAZC	297.3	249.1	200	15	60		
NMPhA	137.1	107	200	10	45		
NMBA	147.1	117.1	200	9	50		
MNP	130.1	100.1	200	15	40		
NpyR	101.1	55.1	200	20	40		

- Preparation of Individual Impurity Standard stock solution.
- Sensitivity solution: 1.0 ng/mL each of above listed N-nitrosamine impurities in Diluent.
- Standard solution: 6.0 ng/mL each of above listed N-nitrosamine impurities in Diluent.
- Sample solution: 50 mg/mL of drug substance prepared as follows. Transfer 250 mg of drug substance into a suitable container. Add 5.0 mL of Diluent and vortex until fully dispersed or dissolved. Pass the solution through a suitable filter of 0.22 µm pore size. Use the filtrate for analysis.
- [NOTE— Divert the API from the MS source during the elution.] Suitability requirements.
- Relative standard deviation: NMT 20.0% from 6 replicate injections, Standard solution.
- Signal-to-noise ratio: NLT 10, Sensitivity solution.
- Analysis: Standard solution and Sample solution.
- Calculate the concentration, in ppm, of each specified nitrosamine impurity in the portion of drug substance taken.
- Result = (r /r) × (C /C) × 10.
- r = peak response of the individual specified nitrosamine impurity from the Sample solution.
- r = peak response of the corresponding nitrosamine impurity from the Standard solution.
- C = concentration of USP N-Nitrosodimethylamine RS, USP N-Nitrosodiethylamine RS, USP N-Nitrosoethylisopropylamine RS, USP Nitrosodiisopropylamine.
- RS, USP N-Nitrosodibutylamine RS, or USP N-Nitrosomethylaminobutyric Acid RS in the Standard solution (µg/mL).
- C = concentration of the drug substance in the Sample solution (µg/mL).
- Report the nitrosamine impurity concentration in the drug substance in ppm (µg/g).

Typical Chromatographic / Mass conditions by GC-MS for multiple Nitrosamine (NDMA, NDEA, NMEA, N-PYR, 1, 4-DNP and NEIPA) impurities quantification:

Column	DB-WAX (30 m x 0.25 mm x 0.5 µm)		
Oven Program	°C/min	Temperature (°C)	Hold (minutes)
	--	60	1
	10	100	1
	15	240	10
Mode of injection	Split		
Split Ratio	1:2		
Carrier gas	Helium		
Carrier gas Flow	1.5ml/minute		
Inlet temperature	180°C		
Injection volume	1.0 µL		
Diluent	Dichloromethane		
Mass spectrometry conditions			
Ion source	Electron Ionization (EI)		
Ion source temperature	230ºC		
Interface temperature	250ºC		
ACQ Mode	SIM		
Event time	0.30 sec		
Detector gain	+0.4kV		
Detector gain mode	Absolute		
Solvent cut time	4.0 min		
Runtime	25.3 minutes		
SIM transition	NDMA		Ch1-m/z:74
	1,4-Dinitroso piperazine		Ch2-m/z: 84
	NMEA		Ch3-m/z: 88
	NPyR		Ch4-m/z:100
	NDEA		Ch5-m/z: 102
	NEIPA		Ch6-m/z: 116

Typical Chromatographic / Mass conditions by GC-MS for multiple Nitrosamine (NMOR, NDIPA, NDBA, NMIPA and 1-NP) impurities quantification:

Column	ZB-5 (30 m x 0.25 mm x 0.25 µm)		
Oven Program	°C/min	Temperature (°C)	Hold (minutes)
	--	60	1
	10	100	1
	15	240	10
Mode of injection	Split		
Split Ratio	1:2		
Carrier gas	Helium		
Carrier gas Flow	1.5ml/minute		
Inlet temperature	180°C		
Injection volume	1.0 µL		
Diluent	Dichloromethane		
Mass spectrometry conditions			
Ion source	Electron Ionization (EI)		
Ion source temperature	230ºC		
Interface temperature	250ºC		
ACQ Mode	SIM		
Event time	0.30 sec		
Detector gain	+0.4kV		
Detector gain mode	Absolute		
Solvent cut time	4.0 min		
Runtime	25.3 minutes		
SIM transition	NMIPA	Ch1-m/z:102	
	1-Nitrosopiperazine	Ch2-m/z: 115	
	NMOR	Ch3-m/z: 116	
	NDIPA	Ch4-m/z:130	
	NDBA	Ch5-m/z: 158	

- Preparation of Individual Impurity Standard stock solution.
- Sensitivity solution: 50.0 ng/mL each of above listed N-nitrosamine impurities in Diluent.
- Standard solution: 20.0 ng/mL each of above listed N-nitrosamine impurities in Diluent.
- Sample solution: 50 mg/mL of drug substance prepared as follows. Transfer 250 mg of drug substance into a suitable container. Add 5.0 mL of Diluent and vortex until fully dispersed or dissolved. Pass the solution through a suitable filter of 0.22 µm pore size. Use the filtrate for analysis.
- [NOTE— Divert the API from the MS source during the elution.] Suitability requirements.
- Relative standard deviation: NMT 20.0% from 6 replicate injections, Standard solution.
- Signal-to-noise ratio: NLT 10, Sensitivity solution.
- Analysis: Standard solution and Sample solution.
- Calculate the concentration, in ppm, of each specified nitrosamine impurity in the portion of drug substance taken.
- Result = (r /r) × (C /C) × 10.
- r = peak response of the individual specified nitrosamine impurity from the Sample solution.
- r = peak response of the corresponding nitrosamine impurity from the Standard solution.
- C = concentration of USP N-Nitrosodimethylamine RS, USP N-Nitrosodiethylamine RS, USP N-Nitrosoethylisopropylamine RS, USP Nitrosodiisopropylamine.
- RS, USP N-Nitrosodibutylamine RS, or USP N-Nitrosomethylaminobutyric Acid RS in the Standard solution (µg/mL).
- C = concentration of the drug substance in the Sample solution (µg/mL).
- Report the nitrosamine impurity concentration in the drug substance in ppm (µg/g).

4.5 Recommendations – for specific Standard Columns, Reagents or any other items.

For GCMS:

- Replace the glass wool and inlet septa before start the analysis and kept the GC column for conditioning at least 1 hour and ensure mass ionization source should be cleaned, air and water check.
- Use Headspace or mass grade solvents for sample preparations.
- Use freshly opened bottle of solvents for sample preparations as it minimizes to exposure to environment may lead to oxidize or absorb any other solvents.
- Minimum of 3 hours system stabilization is required when gas cylinder was replaced and column change.
- Suggest to use Shimadzu make Headspace vial (it contains round bottom flat) while GCMS of Shimadzu make is in use.
- During Headspace method development, evaluate the impact on small quantity of water placed in diluent (i.e., DMSO: H2O, 9:1) to increase the sensitivity of the analyte where limits are very low.

For LCMS:

- Use mass grade solvents and reagents for mobile phase and sample preparations.
- Column should be stabilized with mobile phase.
- Clean source/cone plate before start the long sequence of during method development/validation or any critical batch analysis.
- Evaluate the addition of Ammonia or formic acid (typically 0.05 to 0.1%) in diluent to optimize the recovery.

How to handle potential interferences encountered during method development

For LCMS:

- Needle wash should be select as mobile phase or where sample solubility is more to address RSD failures due to injector
- As the Mass technique is highly selective, any extraneous peak is observed in other retention time can be ignore.
- Ensure the absence of carry over, from the response of a blank injection after analysis of an appropriate standard solution, due to increase in sensitivity of recent LCMS/MS instruments and the use of wide calibration ranges.

- Provide sufficient hold time at the final conditions of a gradient.

- Eliminate the memory effects due to elevated background from MS contamination by ensuring the temperatures in the sprayer and source are set high enough for efficient desolvation.

- Establish the test sample concentrations as much as low concentration based on the desired sensitivity to achieve the good recoveries

- Impurity primary stock solutions should be separated from test sample solution preparation area to avoid contamination/carryover in test solution.

For GCMS:

- Don't use higher temperature (more than 250°C) for Wax column during GCMS method it will lead to column bleed and base line issues and may impact the precision/recovery at low level peaks.

- Replace the glass wool before start the analysis to avoid the RSD issues from non-uniform vaporization of analyte at glass wool due to previous/long injections carryover.

- Syringe should be cleaned and ensure piston operation particularly when Methylene chloride or n-Hexane used as diluent.

- Select the SIM ion very carefully during method development (i.e., if m/z 40 to 60 selected as SIM ion there as a possibility of interferences from other impurities, solvents/matrix, try to avoid to select the SIM ion below m/z 60 based on the analyte mass spectrum/fragments).

5 Abbreviations

AI	:	Acceptable Intake
API	:	Active Pharmaceutical Ingredient
ARB	:	Angiotensin II receptor blocker
CAPA	:	Corrective and Preventive action
CEP	:	Certificate of Suitability
DP	:	Drug Product
EDQM	:	European Directorate for the Quality of Medicines and HealthCare
EMA	:	European Medicines Evaluation Agency
FDA	:	Food and Drug Administration
FDF	:	Finished Dosage Form
FDP	:	Finished drug product
GMP	:	Good Manufacturing Practice
ICH	:	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
KSM	:	Key Starting Material
LOD	:	Limit of Detection
LOQ	:	Limit of Quantitation
MAH	:	Marketing Authorization Holder
MDD	:	Maximum Daily Dose
MRM	:	Multiple Reaction Monitoring
PPB	:	Parts per billion
PPM	:	Parts per million
SIM	:	Selected-ion monitoring chromatogram
TTC	:	Threshold of Toxicological Concern

6 Annexure(s)

1. KSM Risk assessment.
2. Intermediate Drug Substance Risk assessment.
3. Excipients Risk assessment.
4. Risk assessment for Drug Product.

Appendix I
KSM
Risk Assessment

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS			
Product Name & Code			
Document No.		Version No.	

1.0 OBJECTIVE

The objective of this assessment is to evaluate the possibility of presence, detect and prevent unacceptable level of Nitrosamine Impurities.

The assessment also considers the conditions which may introduce Nitrosamine Impurities in Key starting materials.

2.0 SCOPE

This Risk assessment is applicable to the <Product Name with Stage Code> manufactured at <Name of the Company>.

3.0 ROUTE OF SYSNTHESIS

Rout of synthesis of <Name of Product (Including all the stages)> shall be reviewed and shall be attached as an attachment.

4.0 FORMATION OF NITROSAMINE IMPURITIES

Route of synthesis for formation of Nitrosamine impurities shall be provided.

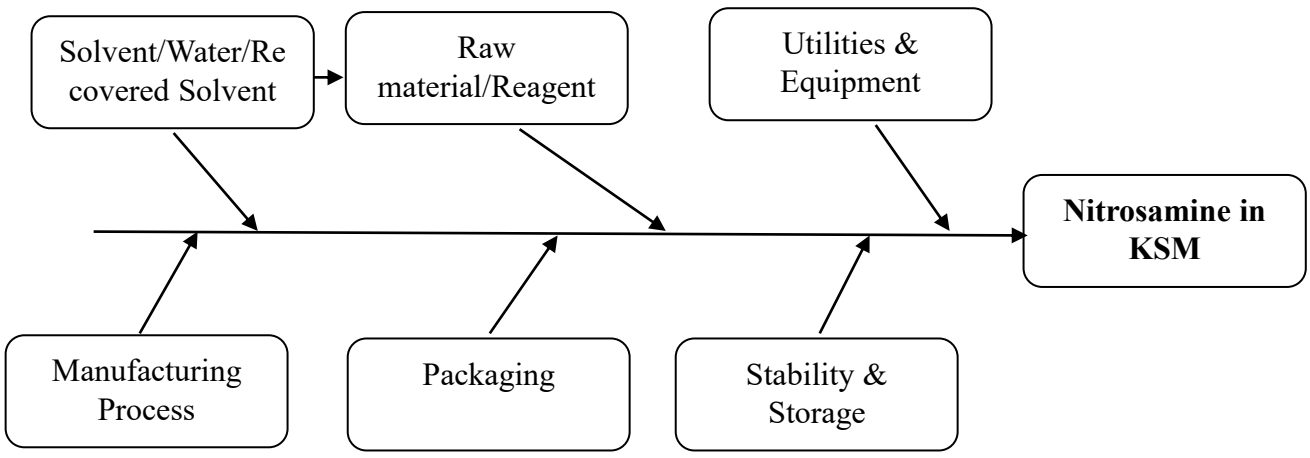
4.1 CONSIDERATION FOR FORMATION OF NITROSAMINE IMPURITIES

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS			
Product Name & Code			
Document No.		Version No.	

5.0 RISK ASSESSMENT APPROACH

To identify the potential source of Nitrosamine impurities that may find their way into Key Starting Materials.

- Raw materials.
- Utilities.
- Manufacturing Process.
- Container Closure.
- Storage & Stability



Note: The risk assessment can be prepared as FMEA approach or by checklist based.

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.1	Whether the Sodium Nitrite, Nitrate (NO3-) or other Nitrosating agents potentially present in the input materials (any raw materials, solvents, reagents, catalysts, water and steam, auxiliary materials such as filter materials, gaskets, and silica gel or work-up reagents) and intermediates? (Based on the review of the Route of synthesis) If yes, Provide the details in which input material Nitrite or Nitrate can be present.								
5.1.1	Review of Rout of synthesis								
5.1.2	**List of Solid Raw materials used in the process**								
	RM-1								
	RM-2								
5.1.3	**List of Solvents used in the process**								
	Solvent-1								
	Solvent-2								
5.1.4	**List of Cleaning Solvents/Agents**								
	Solvent-1								
	Solvent-2								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.1.5	Reagents (Specify the Name)								
5.1.6	Catalysts (Specify the Name)								
5.2	Water (Process/Purified etc.)								
5.2.3	Is chloramine formed as by-product in your water treatment and as a consequence can chloramine be present in the water used for cleaning or as part of the production process? Note: chloramine is known to promote degradation of some relevant API structures to NDMA								
5.2.2	Is potable water (recycled or not) is used in the manufacturing process where any nitrosating agents are used? If recycled water is used, comment on the potential for cross-contamination								
5.3	Equipment								
5.3.1	Usage of Uncleaned equipment								
5.4	Utilities								
5.4.1	Nitrogen Gas								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.4.2	Compressed air								
5.5	List of Primary Packaging Materials								
	Primary Packaging Material - 1								
5.6	Stability and Storage								
5.7	Usage of any Secondary or Tertiary amines/ Quaternary ammonium salts and Amides in the manufacturing process?								
5.8	Whether the usage of Sodium Nitrite / any other Nitrites used in presence of secondary/Tertiary amines/Quaternary amide at the same step or in previous steps? If Yes, Identify the possibility of Nitrosamine impurities:								
5.9	Do you use recycled solvents, reagents and catalysts in the manufacturing process? If so, how do you control and monitor the waste streams sent for recovery, for the presence of Nitrosating agents and amines:								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.10	The recycling process of solvents, reagents and catalyst does involve the quenching step with Nitrous acid? If yes, the specification of such recycled solvents, reagents and catalysts does include the test for Nitrosamines? Note: Examples of recycled materials observed to be contaminated with nitrosamines include Orthoxylene and tributyltin chloride (used as a source of tributyltin azide). It has also been suggested that N, N-dimethylformamide (DMF) could be contaminated in this way.								
5.11	The recycling process of solvents, reagents and catalysts include the aqueous washes or distillations i.e. principles of boiling point / solubility properties If yes, whether the boiling points or solubility properties of the solvents, reagents and catalysts under recycling, are same with that of Nitrosamine impurities? If so, whether the specification of recycled solvents, reagents and catalyst include the test for Nitrosamine content?								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.12	Does your manufacturing process involve the usage of recovered solvents / reagents/ catalysts? If yes, the recovery process is performed in-house or is outsourced?								
	If the recovery process performed in-house, whether the equipment is dedicated to process the recovered solvents only for one product or multiple products?								
	If it is for multiple products, whether the mfg. process of other products for which, the solvents are recovered with common equipment, all the other products are evaluated for Nitrosamine impurities through the steps detailed above. Note: This check shall be applicable even the recovery process is outsourced as stated above.								
5.13	Whether the third party used for recovery of solvents, catalysts and reagents is qualified through the evaluation process w.r.to potential possibility of formation and control over the Nitrosamine impurity formation								
	If Yes, elaborate the evaluation process and established criteria:								
	If No, the third party shall be qualified through the onsite audit considering the checks detailed above.								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.14	The evaluation of the third-party recovery process, include the review of line clearance/ change over process between handling of the products from different customers?								
5.15	Whether the product (KSM) upon degradation yields nitrosation reagent / dialkylamines? If yes, please specify the impact of these degradation products on formation of nitrosamine impurities								

Note : Declaration shall be taken from Manufacturer/Vendor to review and evaluated the possibility for the out sourced materials wherever applicable.

6.0 POSSIBLE NITROSAMINE IMPURITIES

List all nitrosamine impurities identified from above risk assessment, in tabular form with actual compound names and structural formulae:

S.No.	Nitrosamine Impurity Name	Structural Formula	Specification Limit

7.0 CONTROL STRATEGY

7.1 Risk mitigation plan shall be summarised, if a nitrosamine risk is identified based on the above (Section 5.0 & 6.0) risk evaluation.

7.2 The control strategy shall be identified and implemented based on which ICH M7 Option 1-4 control strategy for each identified nitrosamine.

7.3 Analytical test results and validated analytical methods shall be provided as risk mitigation and control strategy.

7.4 If there is likely to be of formation of Nitrosamine impurities and the obtained between LOD and LOQ, 3 lots of final product and periodical batches and in case of changes, shall be tested as per the USP or equivalent validated analytical method.

7.5 If there is a possibility of formation of Nitrosamine impurities and obtained results are above LOQ value, these impurities shall be part of specification.

8.0 CONCLUSION

9.0 REFERENCES (IF ANY)

10.0 ABBREVIATIONS (IF ANY)

11.0 APPROVAL

DEPARTMENT	NAME	SIGN & DATE
Compiled by		
Reviewed by (cross functional team) *		
R&D/PDL		
ADL		
Production		
Quality control		
Quality assurance		
Process engineering		
Approved by		
Quality assurance		

(*) Indicative based on the Organisation.

Appendix II
Intermediate Drug Substance Risk Assessment

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES			
Product Name & Code			
Document No.		Version No.	

1.0 OBJECTIVE

The objective of this assessment is to evaluate the possibility of presence, detect and prevent unacceptable level of Nitrosamine Impurities.

The assessment also considers the conditions which may introduce Nitrosamine Impurities in Drug substance/ Saleable intermediates/ Drug products.

2.0 SCOPE

This Risk assessment is applicable to the <Product Name with Stage Code> manufactured at <Name of the Company>.

3.0 ROUTE OF SYSNTHESIS

Rout of synthesis of <Name of Product (Including all the stages)> shall be reviewed and shall be attached as an attachment.

4.0 FORMATION OF NITROSAMINE IMPURITIES

Route of synthesis for formation of Nitrosamine impurities shall be provided.

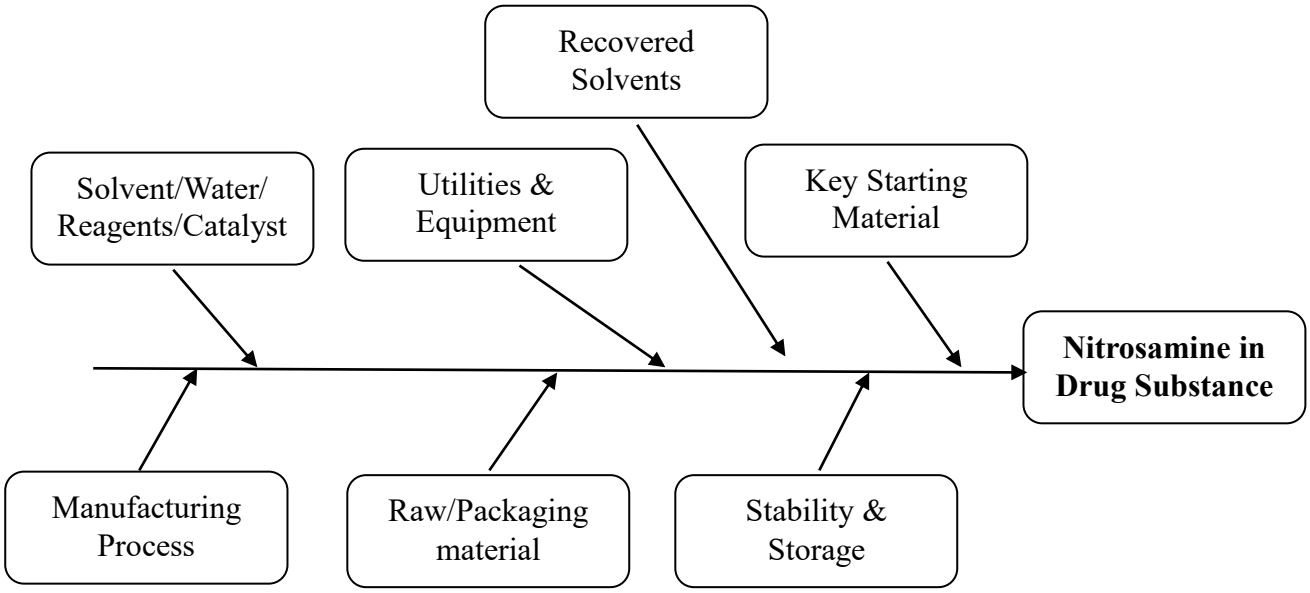
4.1 CONSIDERATION FOR FORMATION OF NITROSAMINE IMPURITIES:

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES			
Product Name & Code			
Document No.		Version No.	

5.0 RISK ASSESSMENT APPROACH

To identify the potential source of Nitrosamine impurities that may find their way into Drug Substance/Intermediate.

- Drug Substance.
- Utilities.
- Manufacturing Process.
- Container Closure.
- Storage & Stability



Note: The risk assessment can be prepared as FMEA approach or by checklist based.

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.1	Whether the Sodium Nitrite, Nitrate (NO3-) or other Nitrosating agents potentially present in the input materials (any raw materials, solvents, reagents, catalysts, water and steam, auxiliary materials such as filter materials, gaskets, and silica gel or work-up reagents) and intermediates? (Based on the review of the Route of synthesis) If yes, Provide the details in which input material Nitrite or Nitrate can be present.								
5.1.1	**Review of Rout of synthesis**								
5.1.2	**List of Key Starting Material used in the process**								
	KSM-1								
	KSM-2								
5.1.3	**List of Solid Raw materials used in the process**								
	RM-1								
	RM-2								
5.1.4	**List of Solvents used in the process**								
	Solvent-1								
	Solvent-2								
5.1.5	**List of Cleaning Solvents/Agents**								
	Solvent-1								
	Solvent-2								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.1.6	Reagents (Specify the Name)								
5.1.7	Catalysts (Specify the Name)								
5.2	Water (Process/Purified etc.)								
5.2.1	Is chloramine formed as by-product in your water treatment and as a consequence can chloramine be present in the water used for cleaning or as part of the production process? Note: chloramine is known to promote degradation of some relevant API structures to NDMA								
5.2.2	Is potable water (recycled or not) is used in the manufacturing process where any nitrosating agents are used? If recycled water is used, comment on the potential for cross-contamination								
5.3	Equipment								
5.3.1	Usage of Uncleaned equipment								
5.4	Utilities								
5.4.1	Nitrogen gas								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.4.2	Compressed air								
5.5	List of Primary Packaging Materials								
	Primary Packaging Material - 1								
5.6	Usage of Printing ink (for foil and Labels)								
5.7	Stability and Storage								
5.8	Usage of any Secondary or Tertiary amines/ Quaternary ammonium salts and Amides in the manufacturing process?								
5.9	Whether the usage of Sodium Nitrite / any other Nitrites used in presence of secondary/Tertiary amines/Quaternary amide at the same step or in previous steps? If Yes, Identify the possibility of Nitrosamine impurities:								
5.10	Do you use recycled solvents, reagents and catalysts in the manufacturing process? If so, how do you control and monitor the waste streams sent for recovery, for the presence of Nitrosating agents and amines:								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS

Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.11	The recycling process of solvents, reagents and catalyst does involve the quenching step with Nitrous acid? If yes, the specification of such recycled solvents, reagents and catalysts does include the test for Nitrosamines? Note: Examples of recycled materials observed to be contaminated with nitrosamines include Orthoxylene and tributyltin chloride (used as a source of tributyltin azide). It has also been suggested that N, N-dimethylformamide (DMF) could be contaminated in this way.								
5.12	The recycling process of solvents, reagents and catalysts include the aqueous washes or distillations i.e. principles of boiling point / solubility properties If yes, whether the boiling points or solubility properties of the solvents, reagents and catalysts under recycling, are same with that of Nitrosamine impurities? If so, whether the specification of recycled solvents, reagents and catalyst include the test for Nitrosamine content?								
5.13	Does your manufacturing process involve the usage of recovered solvents / reagents/ catalysts? If yes, the recovery process is performed in-house or is outsourced?								
	If the recovery process performed in-house, whether the equipment is dedicated to process the recovered solvents only for one product or multiple products?								
	If it is for multiple products, whether the mfg. process of other products for which, the solvents are recovered with common equipment, all the other products are evaluated for Nitrosamine impurities through the steps detailed above. Note: This check shall be applicable even the recovery process is outsourced as stated above.								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN KEY STARTING MATERIALS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.14	Whether the third party used for recovery of solvents, catalysts and reagents is qualified through the evaluation process w.r.to potential possibility of formation and control over the Nitrosamine impurity formation								
	If Yes, elaborate the evaluation process and established criteria:								
	If No, the third party shall be qualified through the onsite audit considering the checks detailed above.								
5.15	The evaluation of the third-party recovery process, include the review of line clearance/ change over process between handling of the products from different customers?								
5.16	Whether the product (API / Intermediate) upon degradation yields nitrosation reagent / dialkylamines? If yes, please specify the impact of these degradation products on formation of nitrosamine impurities								

Note : Declaration shall be taken from Manufacturer/Vendor to review and evaluated the possibility for the out sourced materials wherever applicable.

6.0 POSSIBLE NITROSAMINE IMPURITIES

List all nitrosamine impurities identified from above risk assessment, in tabular form with actual compound names and structural formulae:

S.No.	Nitrosamine Impurity Name	Structural Formula	Specification Limit

7.0 CONTROL STRATEGY

7.1 Risk mitigation plan shall be summarised, if a nitrosamine risk is identified based on the above (Section 5.0 & 6.0) risk evaluation.

7.2 The control strategy shall be identified and implemented based on which ICH M7 Option 1-4 control strategy for each identified nitrosamine.

7.3 Analytical test results and validated analytical methods shall be provided as risk mitigation and control strategy.

7.4 If there is likely to be of formation of Nitrosamine impurities and the obtained between LOD and LOQ, 3 lots of final product and periodical batches and in case of changes, shall be tested as per the USP or equivalent validated analytical method.

7.5 If there is a possibility of formation of Nitrosamine impurities and obtained results are above LOQ value, these impurities shall be part of specification.

8.0 CONCLUSION

9.0 REFERENCES (IF ANY)

10.0 ABBREVIATIONS (IF ANY)

11.0 APPROVAL

DEPARTMENT	NAME	SIGN & DATE
Compiled by		
Reviewed by (cross functional team) *		
R&D/PDL		
ADL		
Production		
Quality control		
Quality assurance		
Process engineering		
Approved by		
Quality assurance		

(*) Indicative based on the Organisation.

Appendix III
Excipients
Risk Assessment

RISK ASSESSMENT FOR N-NITROSAMINE IMPURITIES IN EXCIPIENTS			
Product Name & Code			
Document No.		Version No.	

1.0 OBJECTIVE

The objective of this assessment is to evaluate the possibility of presence, detect and prevent unacceptable level of Nitrosamine Impurities.

The assessment also considers the conditions which may introduce Nitrosamine Impurities in < Excipients>.

2.0 SCOPE

This Risk assessment is applicable to the < Excipients Name with Stage Code> manufactured at <Name of the Company>.

3.0 ROUTE OF SYSNTHESIS

Rout of synthesis of <Name of Excipient> shall be reviewed and shall be attached as an attachment.

4.0 FORMATION OF NITROSAMINE IMPURITIES

Route of synthesis for formation of Nitrosamine impurities shall be provided.

4.1 CONSIDERATION FOR FORMATION OF NITROSAMINE IMPURITIES:

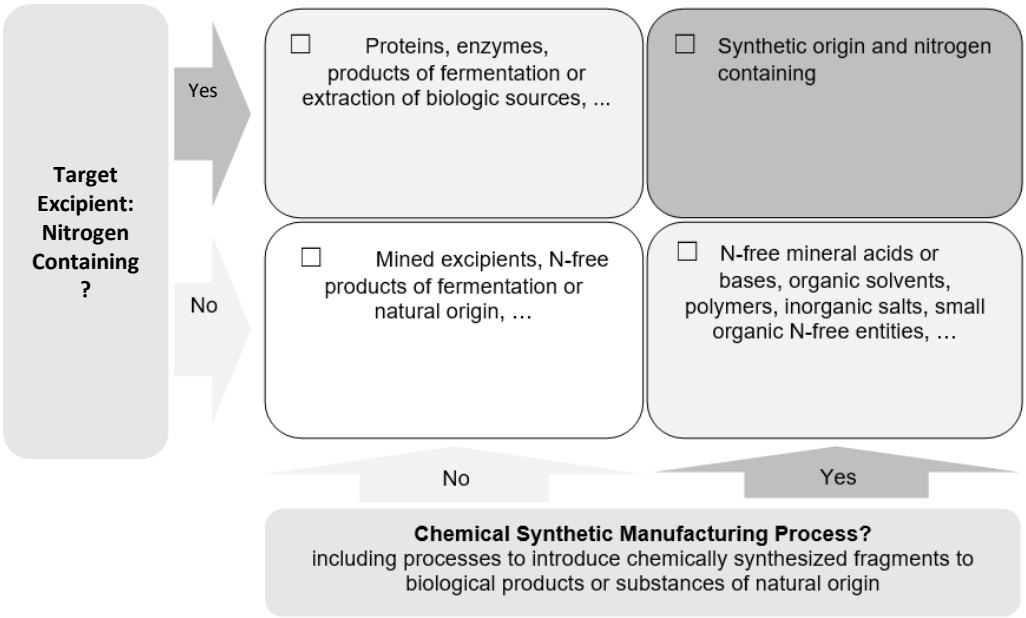
5.0 RISK ASSESSMENT APPROACH

Nitrogen-free materials are considered to be of lower inherent risk for nitrosamine contamination as they are typically manufactured without and do not contain nitrosatable structures. Nitrosamines have been observed in medicinal products with N-containing APIs of chemical synthetic origin. EMA concludes that there is a very low risk of nitrosamines being present as impurities in biological medicinal products, although it can't be completely ruled out.

in this document, "manufacturing process" refers to the manufacturing steps that are outlined in the flow chart of the manufacturing procedure for the mentioned excipient.

RISK ASSESSMENT FOR N-NITROSAMINE IMPURITIES IN EXCIPIENTS			
Product Name & Code			
Document No.		Version No.	

1) Please tick the applicable category based on structure and origin of the excipient in support to evaluate the risk of formation of nitrosamines in the excipient.



2) Is sodium nitrite ($NaNO_2$) or any other nitrite or nitrosating agent:			Information not available
- used in any steps in the manufacturing process as reagents/catalyst?	YES ☐	NO ☐	
- known to be used in the preparation of raw materials or intermediates used in the manufacturing process?	YES ☐	NO ☐	☐
- known to be used in the preparation of reagents/catalysts/processing aids used in the manufacturing process?	YES ☐	NO ☐	☐
- known or likely to be generated during the manufacturing process?	YES ☐	NO ☐	☐
- deliberately added to the process, including components of cell culture media or for fermentation?	YES ☐	NO ☐	☐

RISK ASSESSMENT FOR N-NITROSAMINE IMPURITIES IN EXCIPIENTS			
Product Name & Code			
Document No.		Version No.	

3) Have you analysed, and are the results available for the excipient for: - Nitrites? - Nitrates? - Nitrosamines? If yes, please provide test results for the tested analyte and a general indication of the applied test method and indicate if testing was performed in-house or contracted out.	 YES ☐ YES ☐ YES ☐	 NO ☐ NO ☐ NO ☐	Test result, if available
4) Where water is used in the manufacturing process[3], is it prepared by distillation, by ion exchange or by reverse osmosis? If "No", please inform about the maximum level of - Nitrites - Nitrates	YES ☐ ____ ppm ____ ppm	NO ☐ Not specified ☐ ☐	Not applicable ☐
5) Is there any secondary and/or tertiary amine present in the manufacturing process as[3]: - Raw material? - Intermediate? - Reagent? - Processing aids? - Catalyst / Base? - Solvent? If yes, are those amines present in the - Same - Previous - Subsequent step as any nitrosating agent mentioned in section 2? Information about the chemical name / structure of amine(s):	 YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐	 NO ☐ NO ☐ NO ☐ NO ☐ NO ☐ NO ☐ NO ☐ NO ☐ NO ☐	 Not applicable ☐ ☐ ☐

RISK ASSESSMENT FOR N-NITROSAMINE IMPURITIES IN EXCIPIENTS			
Product Name & Code			
Document No.		Version No.	

6) Is there any amide, primary amine or ammonium salt used or present in the excipient manufacturing process as: - Raw material - Intermediate - Reagent - Processing aid - Catalyst / Base - Solvent - Washing Fluid Information about the chemical name / structure:	 YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐	 NO ☐ NO ☐ NO ☐ NO ☐ NO ☐ NO ☐ NO ☐	
7) Recycled/recovered Solvents: - Are recycled / recovered nitrogen containing solvents used in the manufacturing process?	YES ☐	NO ☐	
8) Multipurpose Equipment: - Is the excipient produced in multipurpose equipment? - In case of multipurpose equipment, is the equipment used for manufacturing of any material involving nitrites, nitrosating agents or material with identified risk of formation of nitrosamines?	YES ☐ YES ☐	NO ☐ NO ☐	 Not applicable ☐

9) **Conclusion**

Please use this field to draw a conclusion about the overall likelihood of the presence of nitrosamines and nitrosating agents.

If “information not available” has been ticked to any option in question 2), please include any additional comments here.

6.0 POSSIBLE NITROSAMINE IMPURITIES

List all nitrosamine impurities identified from above risk assessment, in tabular form with actual compound names and structural formulae:

Sr.No.	Nitrosamine Impurity Name	Structural Formula	Specification Limit

7.0 CONTROL STRATEGY

7.1 Risk mitigation plan shall be summarised, if a nitrosamine risk is identified based on the above (Section 5.0 & 6.0) risk evaluation.

7.2 The control strategy shall be identified and implemented based on which ICH M7 Option 1-4 control strategy for each identified nitrosamine.

7.3 Analytical test results and validated analytical methods shall be provided as risk mitigation and control strategy.

7.4 If there is likely to be of formation of Nitrosamine impurities and the obtained between LOD and LOQ, 3 lots of final product and periodical batches and in case of changes, shall be tested as per the USP or equivalent validated analytical method.

7.5 If there is a possibility of formation of Nitrosamine impurities and obtained results are above LOQ value, these impurities shall be part of specification.

8.0 CONCLUSION

9.0 REFERENCES (IF ANY)

10.0 ABBREVIATIONS (IF ANY)

11.0 APPROVAL

DEPARTMENT	NAME	SIGN & DATE
Compiled by		
Reviewed by (cross functional team) *		
R&D/PDL		
ADL		
Production		
Quality control		
Quality assurance		
Process engineering		
Approved by		
Quality assurance		

(*) Indicative based on the Organisation.

Appendix IV
Risk Assessment for Drug Product

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

1.0 OBJECTIVE

The objective of this assessment is to evaluate the possibility of presence, detect and prevent unacceptable level of Nitrosamine Impurities.

The assessment also considers the conditions which may introduce Nitrosamine Impurities in Drug substance/ Saleable intermediates/ Drug products.

2.0 SCOPE

This Risk assessment is applicable to the <Product Name with Stage Code> manufactured at <Name of the Company>.

3.0 ROUTE OF SYSNTHESIS

Rout of synthesis of <Name of Product (Including all the stages)> shall be reviewed and shall be attached as an attachment.

4.0 FORMATION OF NITROSAMINE IMPURITIES

Route of synthesis for formation of Nitrosamine impurities shall be provided.

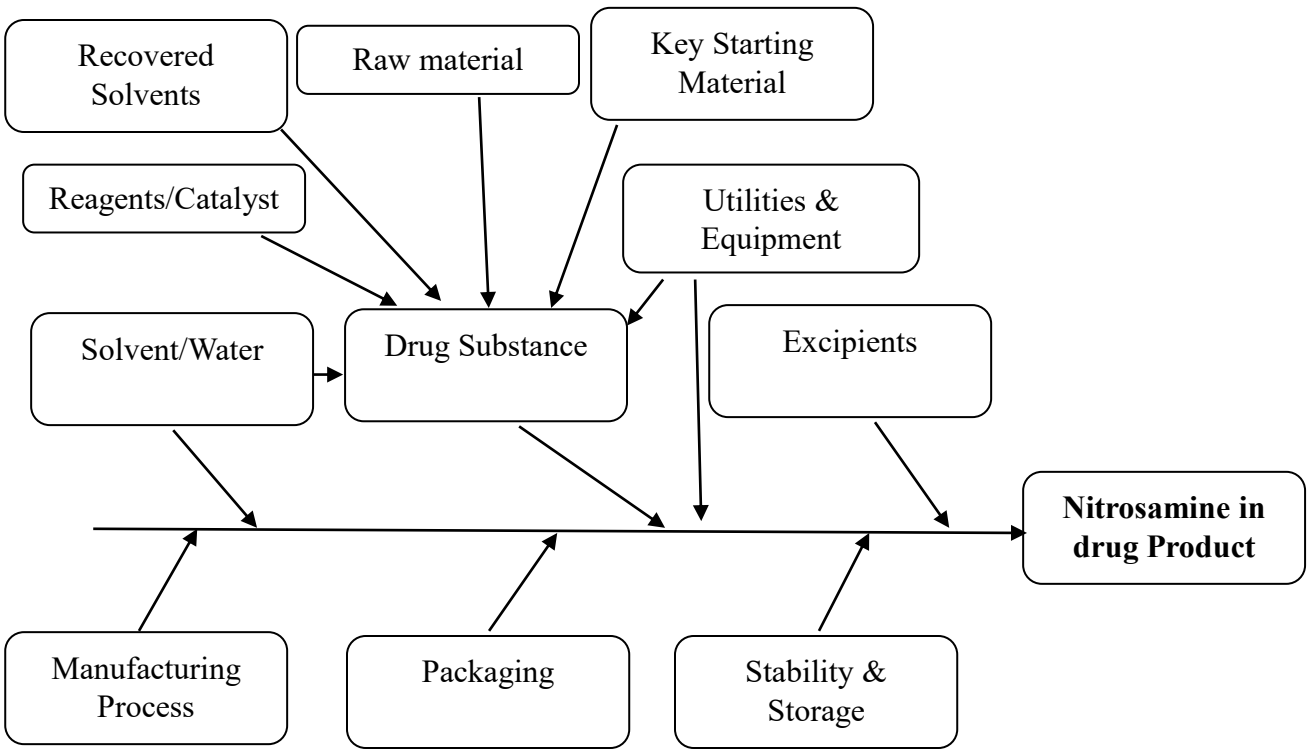
4.1 CONSIDERATION FOR FORMATION OF NITROSAMINE IMPURITIES

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

5.0 RISK ASSESSMENT APPROACH

To identify the potential source of Nitrosamine impurities that may find their way into Drug product.

- Drug Substance.
- Utilities.
- Excipients.
- Manufacturing Process.
- Container Closure.
- Storage & Stability



Note: The risk assessment can be prepared as FMEA approach or by checklist based.

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.1	Whether the Sodium Nitrite, Nitrate (NO3-) or other Nitrosating agents potentially present in the input materials (any raw materials, solvents, reagents, catalysts, water and steam, auxiliary materials such as filter materials, gaskets, and silica gel or work-up reagents) and intermediates? (Based on the review of the Route of synthesis) If yes, Provide the details in which input material Nitrite or Nitrate can be present.								
5.1.1	Review of Rout of synthesis								
5.1.2	List of Key Starting Material used in the process								
	KSM-1								
	KSM-2								
5.1.3	List of Solid Raw materials used in the process								
	RM-1								
	RM-2								
5.1.4	List of Solvents used in the process								
	Solvent-1								
	Solvent-2								
5.1.5	List of Cleaning Solvents/Agents								
	Solvent-1								
	Solvent-2								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.1.6	Reagents (Specify the Name)								
5.1.7	Catalysts (Specify the Name)								
5.2	Water (Process/Purified etc.)								
5.2.1	Is chloramine formed as by-product in your water treatment and as a consequence can chloramine be present in the water used for cleaning or as part of the production process? Note: chloramine is known to promote degradation of some relevant API structures to NDMA								
5.2.2	Is potable water (recycled or not) is used in the manufacturing process where any nitrosating agents are used? If recycled water is used, comment on the potential for cross-contamination								
5.3	Equipment								
5.3.1	Usage of Uncleaned equipment								
5.4	Utilities								
5.4.1	Nitrogen gas								
5.4.2	Compressed Air								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.5	List of Primary Packaging Materials								
	Primary Packaging Material - 1								
5.6	**List of Excipients (Applicable for Drug Products)**								
	Excipient-1								
	Excipient-1								
5.7	Usage of Printing ink (for foil and Labels)								
5.8	Stability and Storage								
5.9	Usage of any Secondary or Tertiary amines/ Quaternary ammonium salts and Amides in the manufacturing process?								
5.10	Whether the usage of Sodium Nitrite / any other Nitrites used in presence of secondary/Tertiary amines/Quaternary amide at the same step or in previous steps? If Yes, Identify the possibility of Nitrosamine impurities:								
5.11	Do you use recycled solvents, reagents and catalysts in the manufacturing process? If so, how do you control and monitor the waste streams sent for recovery, for the presence of Nitrosating agents and amines:								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.12	The recycling process of solvents, reagents and catalyst does involve the quenching step with Nitrous acid? If yes, the specification of such recycled solvents, reagents and catalysts does include the test for Nitrosamines? Note: Examples of recycled materials observed to be contaminated with nitrosamines include Orthoxylene and tributyltin chloride (used as a source of tributyltin azide). It has also been suggested that N, N-dimethylformamide (DMF) could be contaminated in this way.								
5.13	The recycling process of solvents, reagents and catalysts include the aqueous washes or distillations i.e. principles of boiling point / solubility properties If yes, whether the boiling points or solubility properties of the solvents, reagents and catalysts under recycling, are same with that of Nitrosamine impurities? If so, whether the specification of recycled solvents, reagents and catalyst include the test for Nitrosamine content?								
5.14	Does your manufacturing process involve the usage of recovered solvents / reagents/ catalysts? If yes, the recovery process is performed in-house or is outsourced?								
	If the recovery process performed in-house, whether the equipment is dedicated to process the recovered solvents only for one product or multiple products?								
	If it is for multiple products, whether the mfg. process of other products for which, the solvents are recovered with common equipment, all the other products are evaluated for Nitrosamine impurities through the steps detailed above. Note: This check shall be applicable even the recovery process is outsourced as stated above.								

RISK ASSESSMENT FOR NITROSAMINE IMPURITIES IN DRUG SUBSTANCES / SALEABLE INTERMEDIATES /DRUG PRODUCTS			
Product Name & Code			
Document No.		Version No.	

Sr. No.	Review criteria	Potential Source of Impurities			If Yes (Specify the Impurity)	Detectability/ Existing Controls			Mitigation Plan
		YES	NO	NA		YES	NO	NA	
5.15	Whether the third party used for recovery of solvents, catalysts and reagents is qualified through the evaluation process w.r.to potential possibility of formation and control over the Nitrosamine impurity formation								
	If Yes, elaborate the evaluation process and established criteria:								
	If No, the third party shall be qualified through the onsite audit considering the checks detailed above.								
5.16	The evaluation of the third-party recovery process, include the review of line clearance/ change over process between handling of the products from different customers?								
5.17	Whether the product (API / Excipients) upon degradation yields nitrosation reagent / dialkylamines? If yes, please specify the impact of these degradation products on formation of nitrosamine impurities								

Note : Declaration shall be taken from Manufacturer/Vendor to review and evaluated the possibility for the out sourced materials wherever applicable.

6.0 POSSIBLE NITROSAMINE IMPURITIES

List all nitrosamine impurities identified from above risk assessment, in tabular form with actual compound names and structural formulae:

S.No.	Nitrosamine Impurity Name	Structural Formula	Specification Limit

7.0 CONTROL STRATEGY

7.1 Risk mitigation plan shall be summarised, if a nitrosamine risk is identified based on the above (Section 5.0 & 6.0) risk evaluation.

7.2 The control strategy shall be identified and implemented based on which ICH M7 Option 1-4 control strategy for each identified nitrosamine.

7.3 Analytical test results and validated analytical methods shall be provided as risk mitigation and control strategy.

7.4 If there is likely to be of formation of Nitrosamine impurities and the obtained between LOD and LOQ, 3 lots of final product and periodical batches and in case of changes, shall be tested as per the USP or equivalent validated analytical method.

7.5 If there is a possibility of formation of Nitrosamine impurities and obtained results are above LOQ value, these impurities shall be part of specification.

8.0 CONCLUSION

9.0 REFERENCES (IF ANY)

10.0 ABBREVIATIONS (IF ANY)

11.0 APPROVAL

DEPARTMENT	NAME	SIGN & DATE
Compiled by		
Reviewed by (cross functional team) *		
R&D/PDL		
ADL		
Production		
Quality control		
Quality assurance		
Process engineering		
Approved by		
Quality assurance		

(*) Indicative based on the Organisation.

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