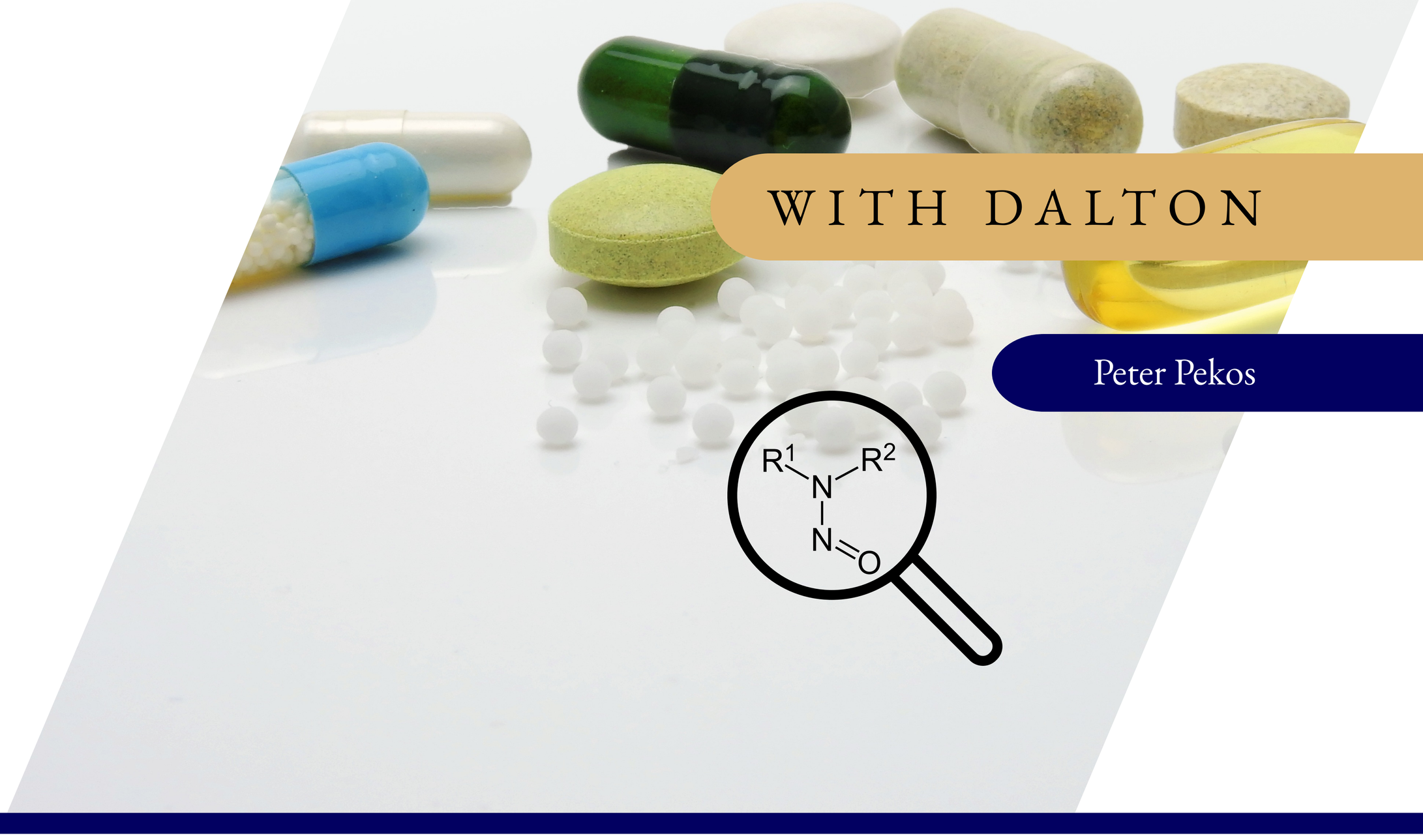
Nitrosamines:
A Review



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Peter Pekos

Company Vision

"To make the impossible possible, Dalton Pharma Services uses its scientific and pharmaceutical expertise to bring customer ideas to life. We develop their new drug products, optimize the synthesis of therapeutic candidates, and manufacture them at the highest level of quality."

Disclaimer

This technical report is intended to provide information to quality and regulatory professionals on best manufacturing practices for nitrosamines and their global regulations. This technical report should be read in conjunction with the relevant laws, regulations, and guidance's that apply to your situation.

FDA inspected, HC approved, & MRA with EMA

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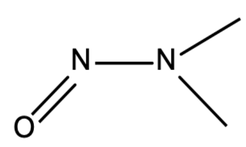
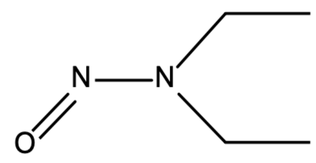
Introduction to Nitrosamines

In 2018, global regulatory agencies first became aware of nitrosamines, such as N-nitrosodimethylamine (NDMA), in commonly prescribed pharmaceuticals like angiotensin receptor blockers, ranitidine, nizatidine, and metformin. Regulations and guidelines around procedures that pharmaceutical industries should take to identify and prevent unacceptable levels of nitrosamines in medicine have since become available. These guidelines highlight the need to implement a comprehensive risk assessment strategy to detect and prevent unacceptable levels of nitrosamine impurities in all chemically synthesised active pharmaceutical ingredients and approved or marketed drug products containing those active pharmaceutical ingredients.

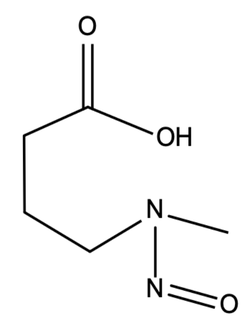
Regulatory agencies have identified seven nitrosamine impurities that could form in drug products: NDMA, N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutanoic acid (NMBA), N-nitrosoisopropylethyl amine (NIPEA), N-nitrosodiisopropylamine (NDIPA), Nnitrosodibutylamine (NDBA), and N-nitrosomethylphenylamine (NMPA).



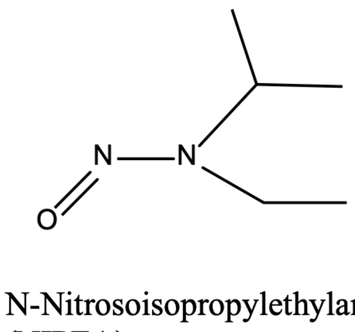
N-Nitrosodimethylamine
(NDMA)



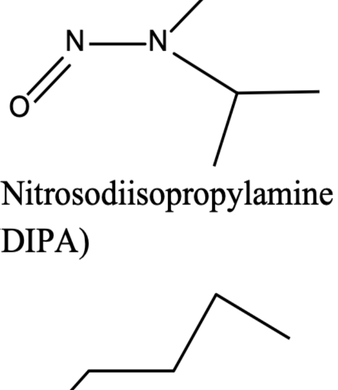
N-Nitrosodiethylamine
(NDEA)



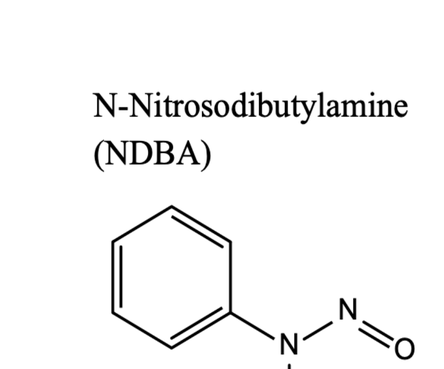
N-Nitroso-N-methyl-4-aminobutyric Acid
(NMBA)



N-Nitrosoisopropylethylamine
(NIPEA)



N-Nitrosodiisopropylamine
(NDIPA)



N-Nitrosodibutylamine
(NDBA)

N-Nitrosomethylphenylamine
(NMPA)

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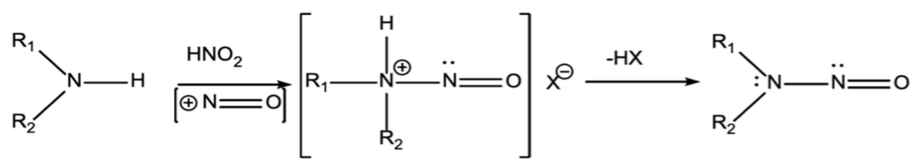
What are Nitrosamines?

Nitrosamines are organic compounds that exist at low levels in our water, food, and small molecule pharmaceuticals. Most nitrosamines are identified as part of a group of high potency mutagenic carcinogens referred to as the “cohort of concern.” Its carcinogenic characteristic is primarily in laboratory animals but may also be involved in the etiology of several human cancers.

Nitrosamines are formed through a common chemical reaction of a secondary or tertiary amine with a nitrosating agent. These reactions can occur during API manufacturing, finished product manufacturing, packaging, or storage.



INTERESTING FACT

Stability testing has found that in certain pharmaceuticals, NDMA levels may increase in time when stored at room temperature.

Sources of amines:

- APIs, API intermediates, starting materials
- Reagents
- Solvents
- Catalysts
- Reaction by-products and degradation products
- Certain non-medicinal ingredients
- Quaternary ammonium salts
- Primary and tertiary amines as they may contain secondary amines
- Amides impurities or amide degradation
- Tertiary amines that may be nitrosated by a dealkylative pathway to produce one or more secondary amines, which may subsequently undergo nitrosation to produce multiple nitrosamines

Nitrosating agent precursors and sources:

- Nitrite ion intentionally used in a manufacturing process (for example, as used in diazotization chemistry or as a reducing agent for azide ion)
- Nitrite present as an impurity in reagents (i.e., sodium azide)
- Nitrogen oxides (i.e., NO, N2O3)
- Nitric acid
- Nitrosyl halides
- Alkyl nitrites and nitro compounds
- Potable/purified water containing nitrite

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Potential and Confirmed Root Causes for The Presence of Nitrosamines in Drug Products:

- Nitrosation of a secondary or tertiary amine during API/ drug product manufacturing or storage, with an inadequate downstream purge of the formed nitrosamine.
- Using a nitrosamine as a starting material or synthetic intermediate, with the incomplete conversion of the nitrosamine and/or insufficient downstream purge.
- A nitrite ion and amine reaction under process alkaline conditions and carbonyl compound catalysis, with insufficient downstream purge.
- Oxidation of a hydrazine functional group in an API, starting material, intermediate, or a reagent to generate nitrosamine, with an inadequate downstream purge.
- Using certain materials such as nitrocellulose or certain types of vulcanisation accelerators (i.e., dithiocarbamate, thiourea, thiruams) in container closure components.
- The use of nitrosamine contaminated material via contaminated vendor-sourced raw material, cross-contamination, or recycled material (i.e., solvents, reagents, catalysts).
- Inadequate operation of a process step meant to purge nitrosamines such as liquid-liquid phase separations.
- Using manufacturing operations that may enhance nitrosamine precursors interaction (for example, wet granulation) or introduce nitrosating agents/precursors (for example, nitrogen oxides during fluid bed drying).
- Inadequate manufacturing process optimization for API reaction conditions such as temperature, pH, or the sequence of adding reagents, intermediates, or solvents.

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Factors to Examine When Determining the Priorities and Order in Which Pharmaceuticals Should Be Assessed:

- Maximum daily dose of the drug product
- Route of administration
- Duration of use
- Indication and consideration of special populations, such as pregnant women and children
- Toxicological profile of the API
- Market aspects such as product availability for sale in a given country and the number of patients being treated with the medicinal product
- New international or domestic information indicating the presence of one or more nitrosamine impurities in an API (or a structurally similar API) or drug product
- The presence of structural elements in the API (i.e., the presence of secondary, tertiary, or quaternary amines and nitrite salts under acidic reaction conditions) or conditions in the manufacturing and packaging processes for the API or drug product, which facilitate nitrosamine formation

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Global Regulations

Health Canada (HC)

Food and Drug Administration (FDA)

European Medicines Agency (EMA)

International regulatory authorities have collaborated to share information and publish guidelines for market authorization holders (MAHs) on the three-step risk evaluation and other analytical procedures for detecting the presence of nitrosamine contaminants in pharmaceutical products. Under these regulations, almost all medicinal products must be tested for nitrosamines, including drug products that are planned for submission or have already been submitted.

1 Evaluate the risk of nitrosamine formation at each stage of the manufacturing process:

This would involve a risk assessment considering probable root causes and sources of impurities formation in each of the following stages:

1. API manufacturing
2. Drug product manufacturing
3. Drug product packaging and storage

If a risk is identified: MAHs should submit the Step 1 response and proceed with Step 2.

If there is no risk: A risk evaluation of the finished product should be completed. Once complete, results should be submitted.

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2 Conduct confirmatory tests (in the case a risk of nitrosamine is confirmed)

There are recommendations on testing and analytical methodologies to conduct confirmatory testing; however, if another method is employed, ensure it is quantitative, adequately sensitive, validated, and performed in a GMP-compliant facility.

If nitrosamines are detected during testing: The root cause should be determined and documented in the report before proceeding to Step 3.

If no nitrosamines are detected: A report should be submitted to (or made accessible to) the appropriate regulatory body.

Nitrosamine	AI Limit (ng/day)	Regulatory Agency Guidance
N-nitroso-methylphenidate	1300	HC
N-nitroso-piperidine	1300	HC
N-nitroso-morpholine(NMOR)	127.0	HC
N-nitroso-duloxetine(NDLX)	100.0	HC
N-nitroso-dimethylamine(NDMA)	96.0	FDA, EMA, HC
N-nitroso-4-(methylamino)butyric acid (NMBA)	96.0	FDA, EMA, HC
1-methyl-4-nitrosopiperazine (MNP)	96.0	HC
N-nitroso-varenicline(NNV)	37.0	HC
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-a]pyrazine(NTTP)	37.0	HC
N-nitroso-1,2,3,6-tetrahydropyridine (NTHP)	37.0	HC
N-nitroso-diethylamine(NDEA)	26.5	FDA, EMA, HC
N-nitroso-diisopropylamine(NDIPA)	26.5	FDA, EMA, HC
NMPA	26.5	FDA, EMA
NIPEA	26.5	FDA, EMA
N-nitroso-ethylisopropylamine(NEIPA)	26.5	HC
N-nitroso-dibutylamine(NDBA)	26.5	HC
N-nitroso-dipropylamine(NDPA)	26.5	EMA, HC
N-nitroso-rasagiline	18.0	HC
nitroso-nortriptyline(NNORT)	8.0	HC
N-methyl-N-nitroso-phenethylamine(NMPEA)	8.0	HC

* Consult the **ICH M7** guideline for guidance on how to determine an AI limit for a nitrosamine impurity that is not listed in the table.
* Consult **ICH S9** if a nitrosamine impurity is identified in a drug product designated for advanced cancer indications
* Consult **ICH Q3A** and **Q3B** if a nitrosamine impurity is identified in a drug product containing an API that is genotoxic at therapeutic concentrations
* If an API or drug product contains several nitrosamine impurities, the total (cumulative) daily exposure should be limited to the nitrosamine having the most conservative AI limit.

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3 Implement and report changes utilized to prevent or decrease nitrosamine impurities

If one or more nitrosamine impurities identified are below the interim acceptable limit: determine the source of impurity and corrective and preventive actions required for corresponding batches.

If one or more nitrosamine impurities identified are above the interim acceptable limit: implement a risk mitigation plan that ensures levels will be consistently within the interim acceptable limits at the end of the product's shelf life

What Can Manufacturers Do to Mitigate Nitrosamine Formation?

- If feasible, avoid nitrosamine-producing reaction conditions; when not possible, demonstrate the process is controlled and capable of reliably eliminating nitrosamine impurities through suitable and robust fate and purge tests.
- If ROS circumstances are likely to result in the formation of nitrosamines, employ bases other than secondary, tertiary, or quaternary amines, where feasible.
- Exercise caution while using amide solvents in ROS such as N,Ndimethylformamide, N,N-dimethylacetamide, and N-methylpyrrolidone.
- During azide breakdown processes, substitute nitrates with other quenching agents.
- Regularly control reaction sequences, processes, and reaction conditions such as pH, reaction time, and temperature.
- Create manufacturing procedures allowing for the removal of nitrosamine contaminants in downstream processing steps.
- API manufacturers should be aware that low quantities of nitrite and nitrosamines may be present in potable water used in API manufacturing due to environmental pollution.

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The Latest Nitrosamine News

FDA recommends alternate methodologies for nitrosamine mitigation

November 2021

The 2020 guidance outlined only one mitigation strategy - the implementation of a supplier qualification program to evaluate potential impurities across excipient suppliers and excipient lots.

In November 2021, the FDA recommended alternative approaches that may be equally or more effective. The first of these approaches is based on in vitro studies on human gastric fluid, indicating that frequently used antioxidants such as ascorbic acid (vitamin C) or alpha-tocopherol (vitamin E) reduce the formation of nitrosamines. Another mitigation strategy involves the use of excipients such as sodium carbonate to change the micro-environment to neutral or basic pH.

For more info click here.

EMA clarifies the management of various nitrosamines

February 2022

The amended guidance adds a new nitrosamine to the list of those that should be tested: N-nitrosodipropylamine (NDPA) with a maximum daily limit of 26.5 parts per million (ppm) and clarifies how to assess the risk of multiple nitrosamines in a drug substance. EMA gives manufacturers two options for calculating nitrosamine impurities when more than one nitrosamine impurity is identified. Manufacturers can either calculate the total daily intake of all identified N-nitrosamines and ensure that these limits do not exceed the acceptance intake (AI) limits of the most potent nitrosamine, or they can ensure that the total risk level does not exceed the International Council for Harmonisation's M7(R1) guideline.

For more info click here.

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Health Canada revises nitrosamines guidance

April 2022

Health Canada has revised its nitrosamines guidance (formerly the nitrosamines Q&A paper). It is available online and has also been distributed to all holders of a drug identification number and a drug establishment licence.

Key additions and changes:

Under General:

- Considerations regarding filing specific applications where nitrosamines have been identified (for example, for supplements and notifiable changes, clinical trial applications).

Under Safety:

- 10 nitrosamines have been added to the list of established acceptable intake (AI) limits under.
- Using the class-specific toxicological concern (TTC) of 18 ng/day for nitrosamines as a default limit when AI is now established.
- Revised guidelines on AI limits where several nitrosamines are discovered in an API or drug product and where drug products fall within the scope of the ICH's S9 guideline or when the API is genotoxic.

Under Quality:

- Probable root causes of nitrosamine presence.
- Information on control strategy alternatives when nitrosamines are present.
- Suggestions on testing a representative number of batches when a risk concern has been discovered and the underlying cause is known.

Resources

1. USP Nitrosamine Impurities
2. EP 2.5.42. N-Nitrosamines in active substances
3. ICH M7 (R1)
4. Health Canada Guidance: Nitrosamine impurities in medications
5. FDA Guidance: Control of Nitrosamine Impurities in Human Drugs *Guidance for Industry*
6. EMA Guidance: Nitrosamine impurities
7. EMA: Questions and answers for marketing authorisation holders / applicants on the CHMP opinion for the Article 5(3) referral

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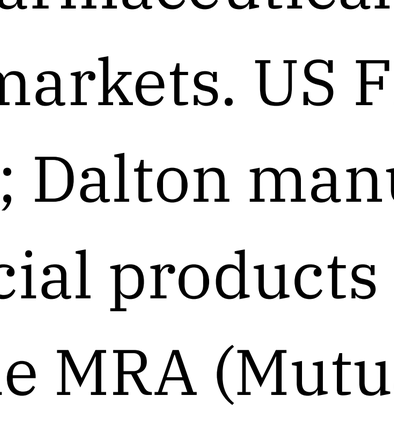
- Synthetic route development
- Feasibility studies & tech transfer
- Process optimization & scale-up
- Scale-up troubleshooting
- Engineering batches
- cGMP API manufacturing



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References

1. EMA. (2020). *Nitrosamine impurities*. European Medicines Agency. https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities

2. FDA. (September 2020). *Control of Nitrosamine Impurities in Human Drugs Guidance for Industry.* Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs

3. Frontiers. (November 2021). *The EU Response to the Presence of Nitrosamine Impurities in Medicines.* Regulatory Science. https://www.frontiersin.org/articles/10.3389/fmed.2021.782536/full

4. HC. (April 2022). *Nitrosamine impurities in medications: Overview.* Health Canada. https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities.html

5. HC. (May 2022). *Nitrosamine impurities in medications: Guidance.* Health Canada. https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities/medications-guidance.html

6. ICH. (2021). *M7 Mutagenic impurities.* International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. https://www.ich.org/page/multidisciplinary-guidelines

7. RAPS. (November 2021). *FDA suggests alternative approaches for nitrosamine risk assessments.* Regulatory Affairs Professional Society. https://www.raps.org/news-and-articles/news-articles/2021/10/fda-suggests-alternative-approaches-for-nitrosamin

8. RAPS. (February 2022). *EMA offers more clarity on controlling multiple nitrosamines.* Regulatory Affairs Professional Society. https://www.raps.org/news-and-articles/news-articles/2022/2/ema-offers-more-clarity-on-controlling-multiple-ni

9. Science Direct. (2014). *Nitrosamines.* Encyclopedia of Food Safety. https://www.sciencedirect.com/topics/earth-and-planetary-sciences/nitrosamine

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