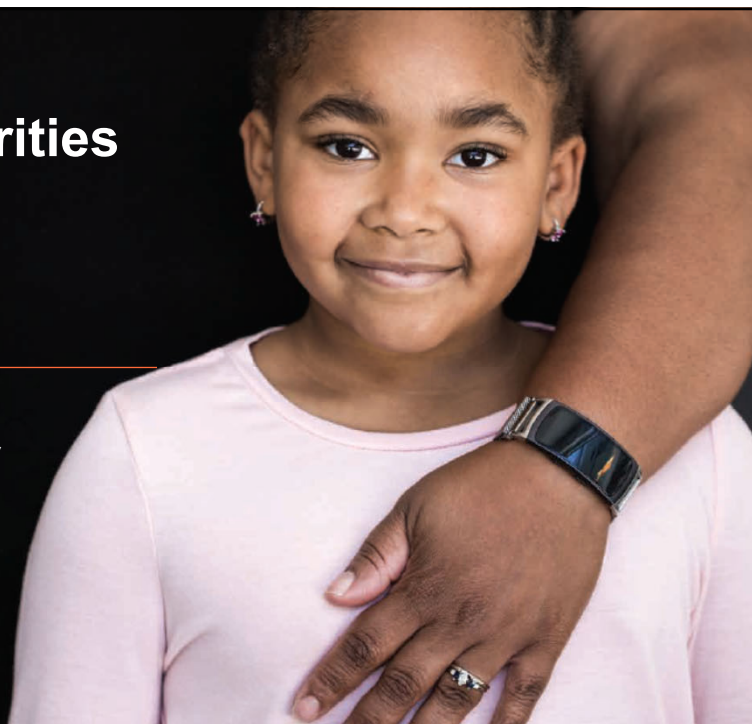


Nitrosamine Impurities An Overview

Naiffer Romero
Scientific Affairs Manager
Sep, 2020



Agenda

- ▶ Background and Regulatory Concerns
- ▶ Nitrosamines Formation & Sources
- ▶ Defining Limits for Impurities
- ▶ Regulatory Approach (Risk & Control)
 - FDA perspective
 - EDQM Lessons learnt
- ▶ Analytical Methods for Nitrosamines Quantification
- ▶ USP Nitrosamines Activities -
 - Proposed General Chapter <1469> outline
 - USP Reference Standards

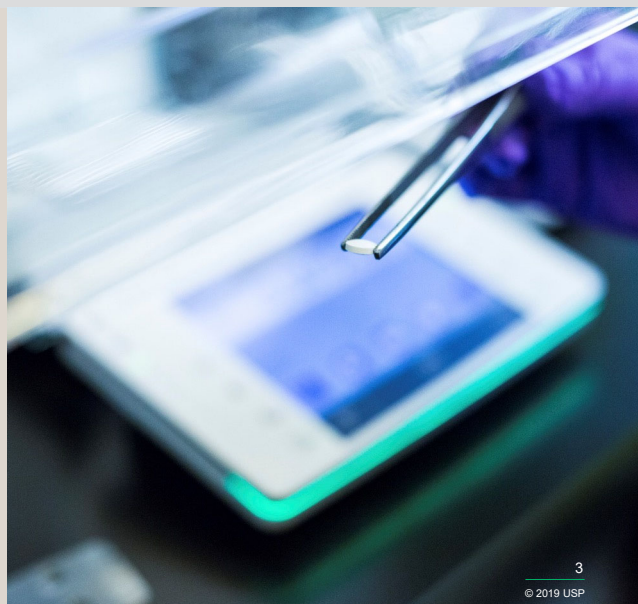


Regulatory Concerns & Awareness



N-Nitrosamine impurities

- ▶ The nitrosamine presence in pharmaceutical products emerged as a public health concern in 2018 after reports that harmful levels of nitrosamine impurity, *N*-nitrosodimethylamine (NDMA), were observed in angiotensin II receptor blockers (ARBs) (Sartan products).
- ▶ Following these reports and after further investigation, agencies issued public health alerts and guidance documents, which have limits, regarding the presence of nitrosamine impurities in several drug products:
 - World Health Organization (WHO),
 - US Food and Drug Administration (FDA),
 - European Directorate for the Quality of Medicines (EDQM), and other agencies.



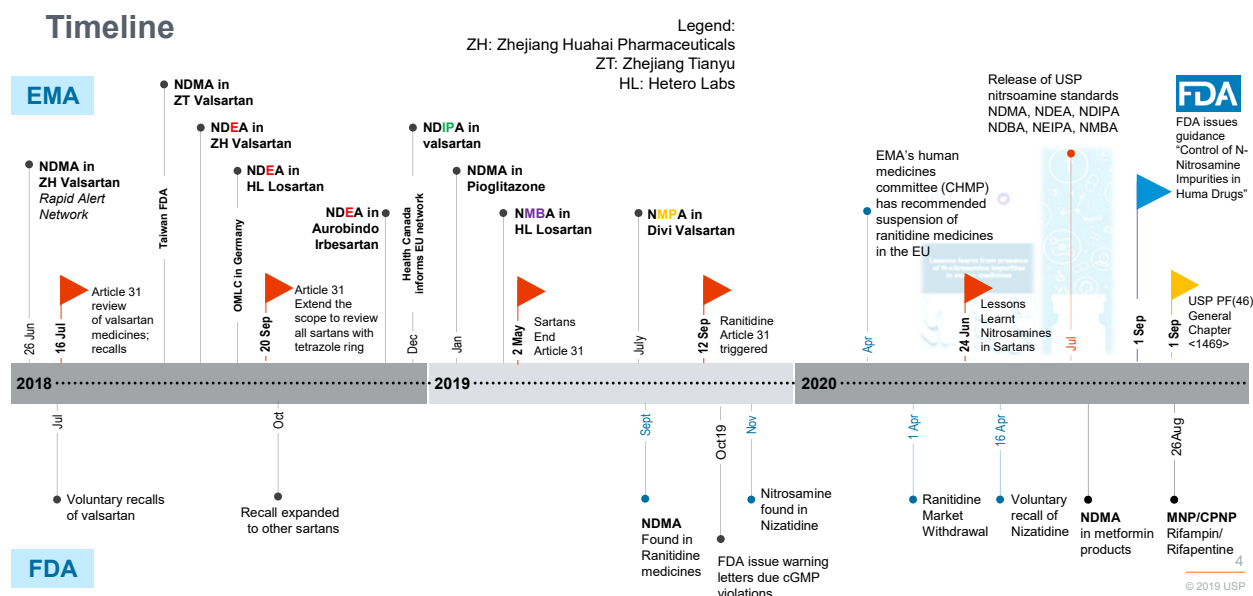
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Regulatory and Other Actions



Timeline



Background



N-Nitrosamine impurities

- ▶ N-Nitrosamines are a class of chemical compounds with the general structure shown in Figure 1. The essential feature of N-nitroso compounds is the N–N=O structure;
- ▶ They are classified as probable human carcinogens (**mutagenic carcinogens** DNA Reactive Impurities referred to as the “cohort of concern” in ICH M7 (aflatoxin-like, N-nitroso-, and alkyl-azoxy compounds

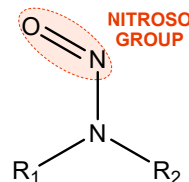
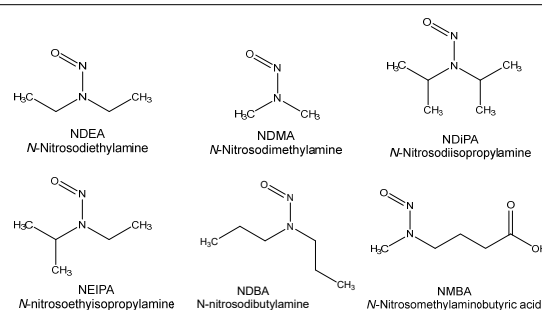


Figure 1. Generic N-nitrosamine structure



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Background



N-Nitrosamine impurities

- ▶ N-Nitrosamines are classified by the ICH M7(R1) Guideline (“Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk”) as Class 1 impurities (“known mutagenic carcinogens”)³

Table 1: Impurities Classification with Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions

Class	Definition	Proposed action for control (details in Section 7 and 8)
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive*, no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5; If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

ICH M7(R1) Guideline “Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk”²

- ▶ Many N-nitrosamines are classified as probable carcinogens (group 2A) by International Agency for Research on Cancer [IARC]^{1,2}

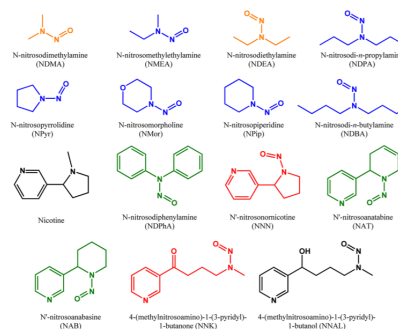


Figure 1. Chemical structures of the target N-nitrosamines, TSNA and nicotine and their respective IARC classifications: (red) group 1, known carcinogens to humans; (orange) group 2A, probable carcinogens to humans; (blue) group 2B, possible carcinogens to humans; and (green) group 3, not classifiable as to its carcinogenicity to humans.^{1,1}

²Farren, J.F. et al. Estimated Exposure Risks from Carcinogenic Nitrosamines in Urban Airborne Particulate Matter. Environ. Sci. Technol. 2015, 49, 9648–9656

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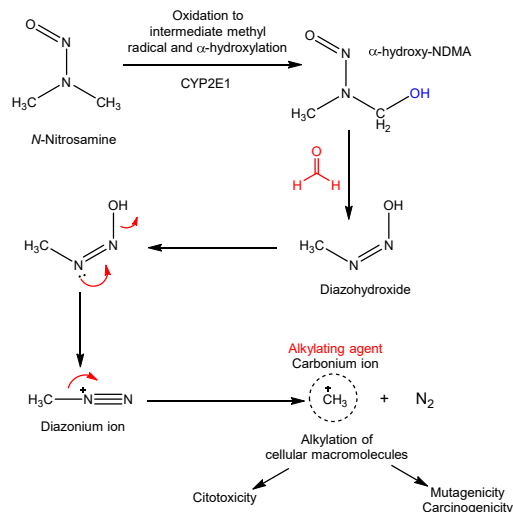
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Background



N-Nitrosamine impurities

- ▶ Nitrosamines are metabolized in liver and the metabolism of some of them can produce DNA reacting agent



Cheung, P.C.W. A Historical Review of the Benefits and Hypothetical Risks of Disinfecting Drinking Water by Chlorination. Journal of Environment and Ecology 2017, Vol. 8, No. 1

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Background

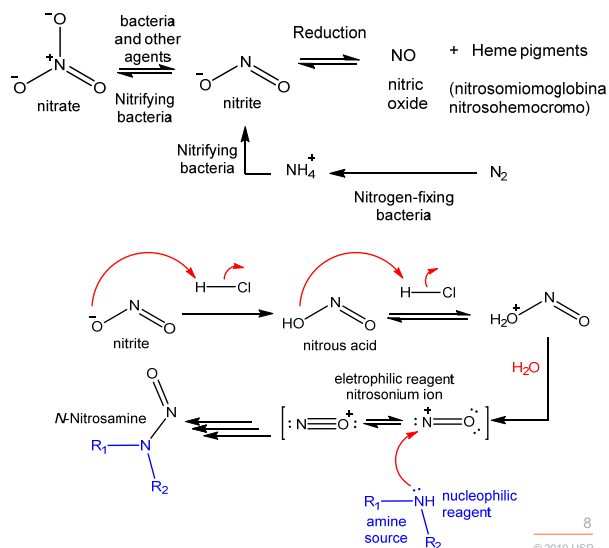


N-Nitrosamine impurities

- ▶ N-Nitrosamines (or their precursors) are common in⁴

- wide variety of foods:
 - cured and grilled meats, bacon,
 - vegetables (raw spinach, lettuce etc)
 - dairy products
- Drinking Water
- manufactured and natural products, such as agricultural chemicals, tobacco, detergents, solvents, drugs, plastics, cosmetics etc.

- ▶ Everyone is exposed to some level of nitrosamines.
- ▶ Human exposure to nitrosamines can result from formation of N-nitroso compounds either in food (even during storage or preparation) or in-vivo.



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References



- [1] International Agency for Research on Cancer; "IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines"; Volume 89, 2007, <https://monographs.iarc.fr/iarc-monographs-on-the-evaluation-of-carcinogenic-risks-to-humans-32/>
- [2] Farren, J.F. et al. Estimated Exposure Risks from Carcinogenic Nitrosamines in Urban Airborne Particulate Matter. Environ. Sci. Technol. 2015, 49, 9648–9656
- [3] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; "ICH Harmonised Guideline - Assessment And Control Of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, M7(R1)"; March 31, 2017,
- [4] Cheung, P.C.W. A Historical Review of the Benefits and Hypothetical Risks of Disinfecting Drinking Water by Chlorination. Journal of Environment and Ecology 2017, Vol. 8, No. 1
- [5] https://www.who.int/medicines/publications/drugalerts/InformationNote_Nitrosamine-impurities/en/
- [6] <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs>
- [7] https://www.ema.europa.eu/en/documents/other/temporary-interim-limits-nmba-dipna-eipna-impurities-sartan-blood-pressure-medicines_en.pdf
- [8] https://www.ema.europa.eu/en/documents/report/lessons-learnt-presence-n-nitrosamine-impurities-sartan-medicines_en.pdf

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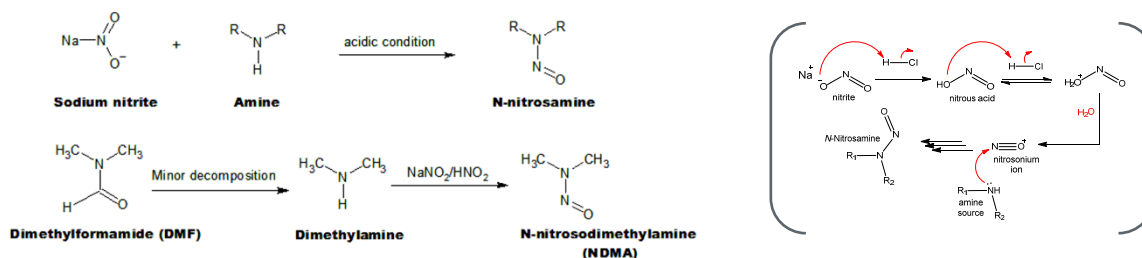
Nitrosamines Formation



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Nitrosamine Formation



- ▶ In general, nitrosamines are formed by the reactions of secondary amines with nitrite in acid conditions
- ▶ Nitrosamine impurities or precursor can be potentially found throughout entire manufacturing process

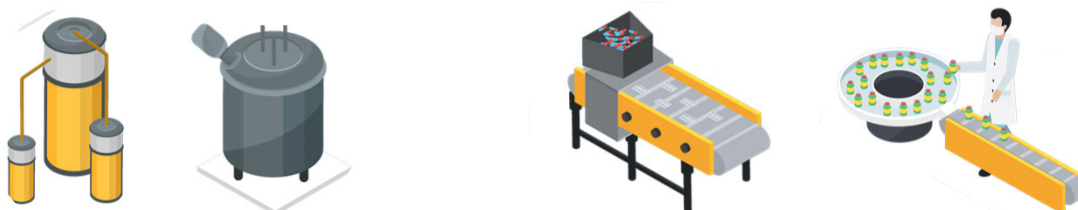
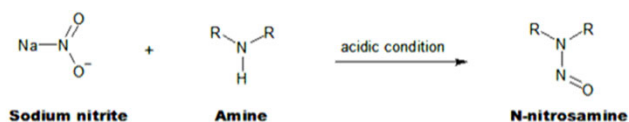
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Nitrosamine Sources



Potential sources of nitrosamine impurities in drug product



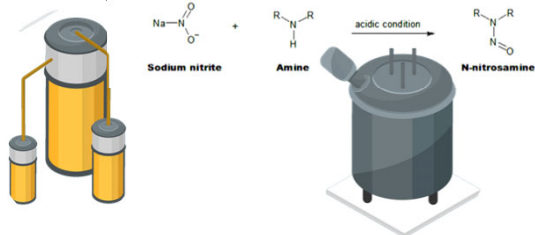
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Nitrosamine Sources



Pre-Drug Product



Sources of Nitrite:

- ▶ Nitrites are sometime used to quench reactions involving azides. In these reactions excess azide is used and the nitrite solutions is used to stop the reaction and quench the excess azide.
- ▶ Nitrites are often present as impurities in other reagents. Sodium nitrite is commonly present in sodium nitrate and in sodium azide
- ▶ Organic nitrites can also give rise to inorganic nitrites under harsh reaction conditions

Sources of Amines:

- ▶ The presence of secondary, tertiary, or even quaternary amines in the chemical synthesis
- ▶ Sources of secondary amines as an impurity in other reagents
- ▶ Sources of secondary amines being formed in the reaction
- ▶ The risk of carryover from one step in the synthesis pathway to another step

Other sources:

- ▶ Water
- ▶ Solvents (Fresh vs Recovered solvents)
- ▶ Contamination in cross over process

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Nitrosamine Sources



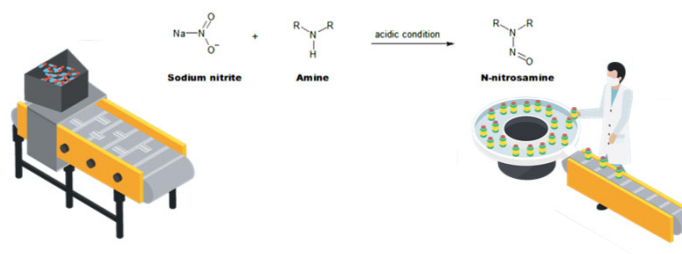
Post-Drug Product

Water

- ▶ Nitrosamine are potential environmental contaminants in water
- ▶ Drug Product manufacturing (Eg. Final rinse, cleaning cycles)
- ▶ Purification process leading to pharmacopeial grade 'Purified Water' should remove organics including Nitrosamines

Degradation Products

- ▶ Short-storage
- ▶ Long-storage



Interaction of API w/ Excipients

- ▶ If API contains amino groups (secondary amines). There is potential for interaction with nitrites in the excipient matrix
- ▶ Unlikely, but sometime Nitrites are used as preservatives or as contaminant

Packaging Process

- ▶ For blister packaging:
 - 1) Printing ink may contain amines,
 - 2) Lacquer used on the aluminum foil may contain nitrocellulose

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Regulatory Approach (Risk & Control)



Defining Limits for Impurities



- ▶ NDMA classified as “Probable carcinogenic to humans” [IARC, WHO]
- ▶ NDMA belongs to *N*-nitroso compounds > “Cohort of Concern” [ICH M7]
- ▶ Acceptable intake (AI) must be derived from specific carcinogenicity data
 - ▶ 50% tumor incidence (TD_{50}) > NDMA: 0.096 mg/kg/day [Carcinogenic Potency Database]
 - ▶ $AI = TD_{50} / \text{Max Daily dose}$
 - ▶ $AI \text{ (NDMA)} = 96 \text{ ng/day} / 320 \text{ mg/day [Valsartan]}$

Source: <https://www.americanpharmaceuticalreview.com/Featured-Articles/561484-Nitrosamines-in-Pharmaceuticals-Toxicity-Risk-Analysis-Chemistry-and-Test-Methods/>

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FDA Approach	EMA Approach
<ul style="list-style-type: none"> All chemically synthesized APIs in human drugs (including drug products) 	All human medicinal products, <ul style="list-style-type: none"> Chemically synthesized APIs And Biological products
<ul style="list-style-type: none"> Risk-based approach 	<ul style="list-style-type: none"> Risk-based approach
<ul style="list-style-type: none"> AI limits for NDMA, NDEA, NMBA, NMPA, NIPEA, NDIPA 	<ul style="list-style-type: none"> Limits for NDMA, NDEA, EIPNA, DIPNA, NMBA, MeNP, and NDBA

Nitrosamine	AI Limit (ng/day) ^{1,2}
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

² 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 ($\text{ppm} = \text{AI (ng)} / \text{MDD (mg)}$).

N-Nitrosamine (CAS number)	ng/day***
NDMA* (62-75-9)	96.0
NDEA*(55-18-5)	26.5
ElPNA**(16339-04-1)	26.5
DiPNA**(601-77-4)	26.5
NMBA*(61445-55-4)	96.0
MeNP**(16339-07-4)	26.5
NDBA**(924-16-3)	26.5

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FDA Recommendations



Assess the risk

- In a timely manner, within 6 months (March 2021)
- Based on prioritization of drugs

Conduct confirmatory testing

- When there is any risk for the presence of nitrosamine
- Specific and sensitive analytical methods

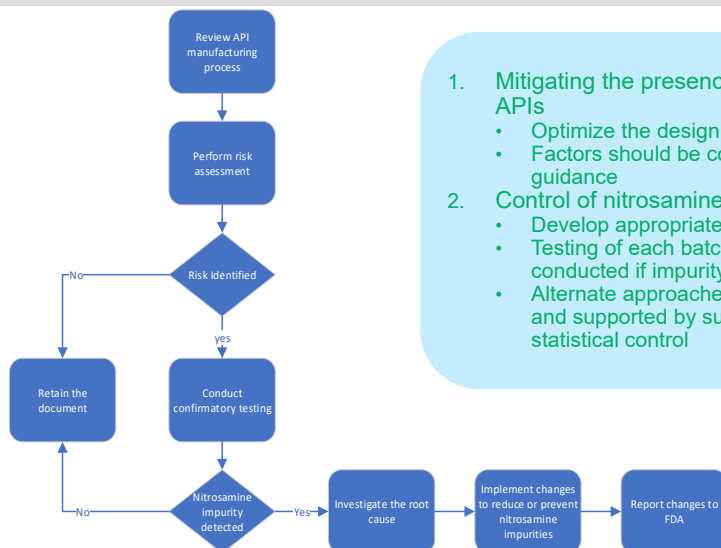
Report changes

- DMF amendments or drug applications
- Within 3 years of publication of guidance (Sept. 2023)

Source: <https://www.fda.gov/media/141720/download>

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FDA Recommendations for API Manufacturers

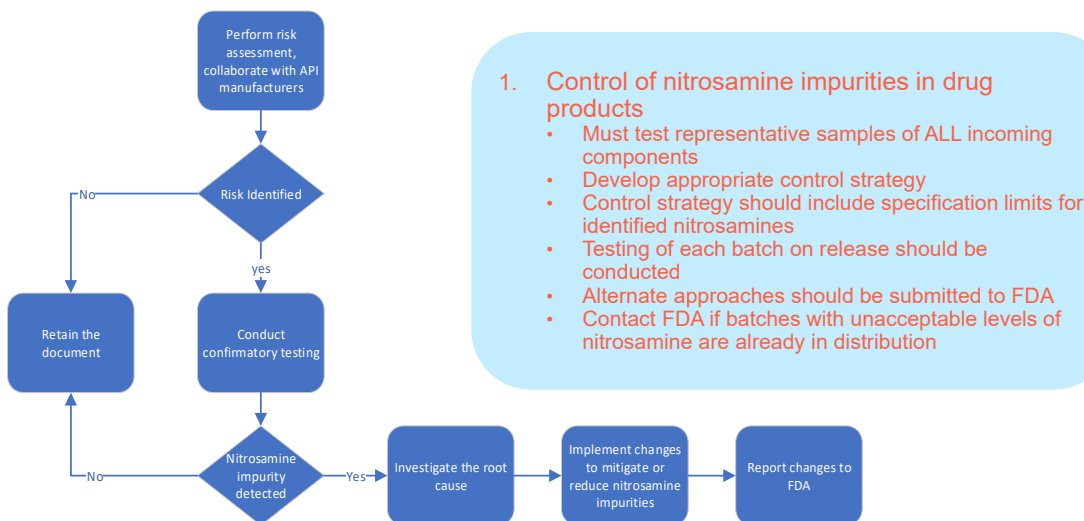


1. Mitigating the presence of nitrosamine impurities in APIs
 - Optimize the design of the manufacturing process
 - Factors should be considered are provided in the guidance
2. Control of nitrosamine impurities in APIs
 - Develop appropriate control strategy
 - Testing of each batch on release should be conducted if impurity detected above the LOQ
 - Alternate approaches should be submitted to FDA and supported by sufficient evidence of adequate statistical control

Source: <https://www.fda.gov/media/141720/download>

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FDA Recommendations for DP Manufacturers

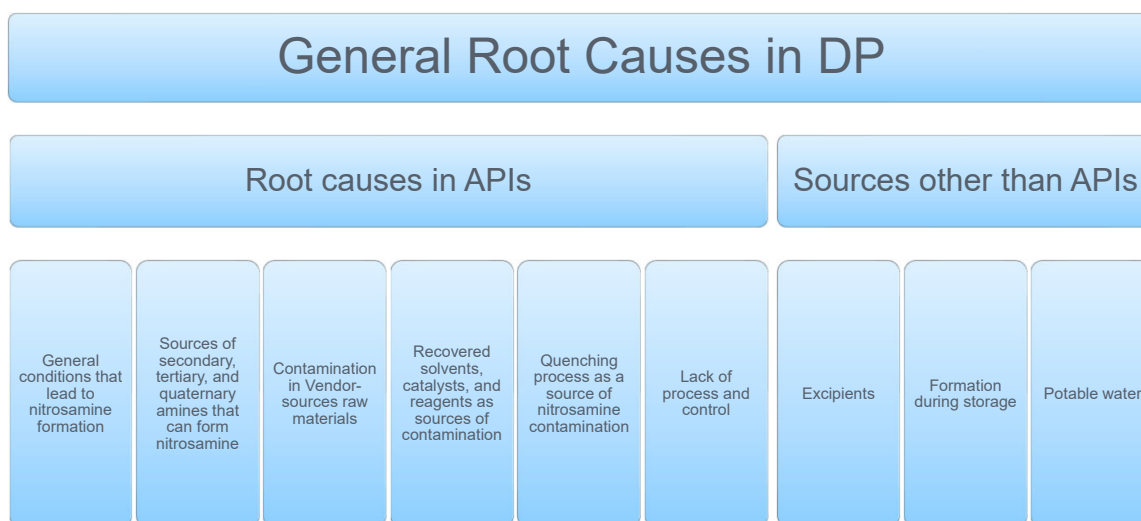


Source: <https://www.fda.gov/media/141720/download>

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General Root Causes Identified in FDA Guidance



Source: <https://www.fda.gov/media/141720/download>

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EMA Guidance for Marketing Authorization Holders



- Marketing authorization holders should
 - Review their manufacturing processes for all products - chemically synthesized or biological active substances
 - Identify and mitigate the risk of presence of nitrosamine impurities
- The approach for risk evaluation/risk assessment should cover manufacturing processes of active substance and finished product in consideration of the root-causes, and subsequent confirmatory testing in the finished product, in case a risk is identified

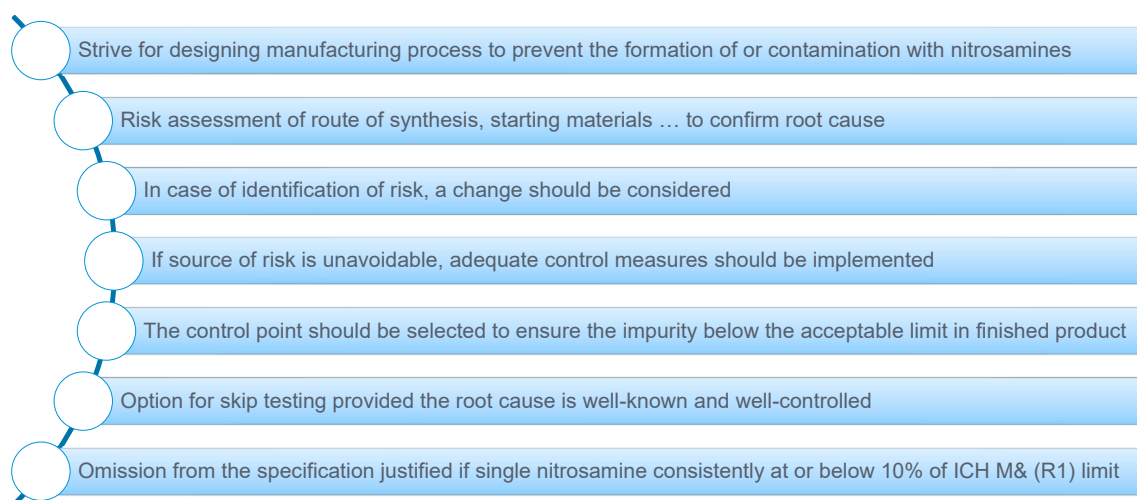


Source: <https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities#lessons-learned-with-sartan-medicines-and-recommendations-section>

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EMA Recommended Mitigation Strategies



Source: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf

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Analytical Procedures for Nitrosamines Quantification



Regulatory Actions



Published Limits for Nitrosamines (FDA/EMA)

Limits for NDMA, NDEA, NMBA, NEIPA and NDIPA in Angiotensin II Receptor Blockers (ARBs): FDA⁶/EMA^{7,8}

Drug	Maximum Daily Dose (mg/day)	Acceptable Intake (ng/day)*					Acceptable Intake (ppm)* (Interim Limits) - FDA/EMA				
		NDMA	NDEA	NMBA	NEIPA**	NDIPA**	NDMA	NDEA	NMBA	NEIPA**	NDIPA **

PLEASE REFER TO YOUR REGULATORY AUTHORITY
FOR CURRENT OFFICIAL NITROSAMINES LIMITS

* These values are based on a drug's maximum daily dose as reflected in the drug label (FDA is temporarily not objecting to losartan with NMBA below 9.82 ppm remaining on the market)

**EMA set Temporary limits for NDMA and NDEA impurities during Article 31 review (20Sep18) and applying the same principles used to set limits for NDMA and NDEA, established limits for three additional N-nitrosamines: EIPNA, DIPNA and NMBA (20Aug2019).

***FDA is temporarily not objecting to losartan with NMBA below 9.82 ppm remaining on the market

- Immediate action: batches containing nitrosamines above the limits, at whatever quantifiable level, will not be allowed on the market
 - For all N-nitrosamines, the MAH must ensure a control strategy is in place in drug substance batches used for their drug products Specifications must include the limits
- Within 2 year: Manufacturing processes to be reviewed for the potential risk of formation of N-Nitrosamines and to be changed as necessary to minimize nitrosamine contamination as much as possible: Levels of Nitrosamines should be "not quantifiable" (< 0.03 ppm).

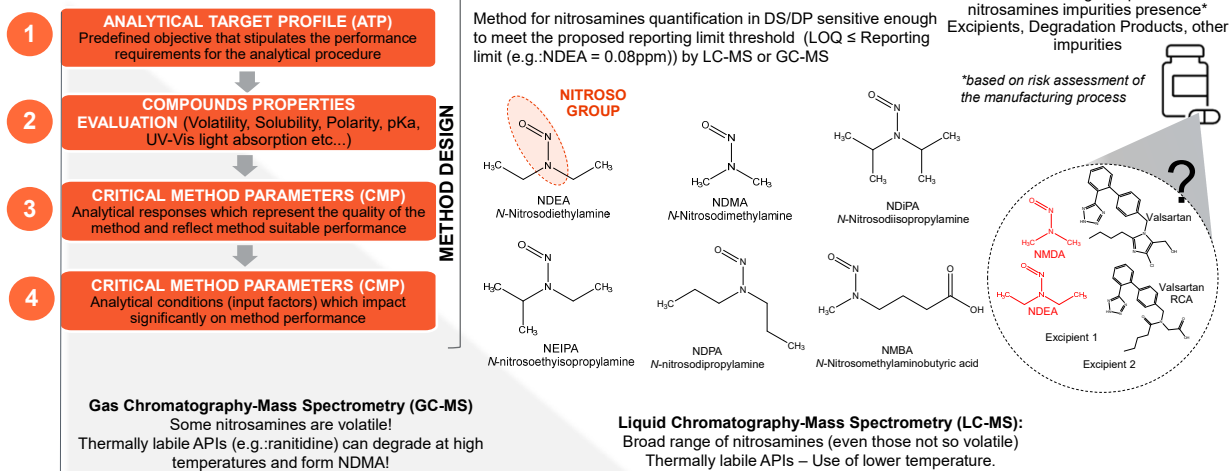
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Analytical Procedure for Nitrosamines Quantification



Analytical Procedure Development



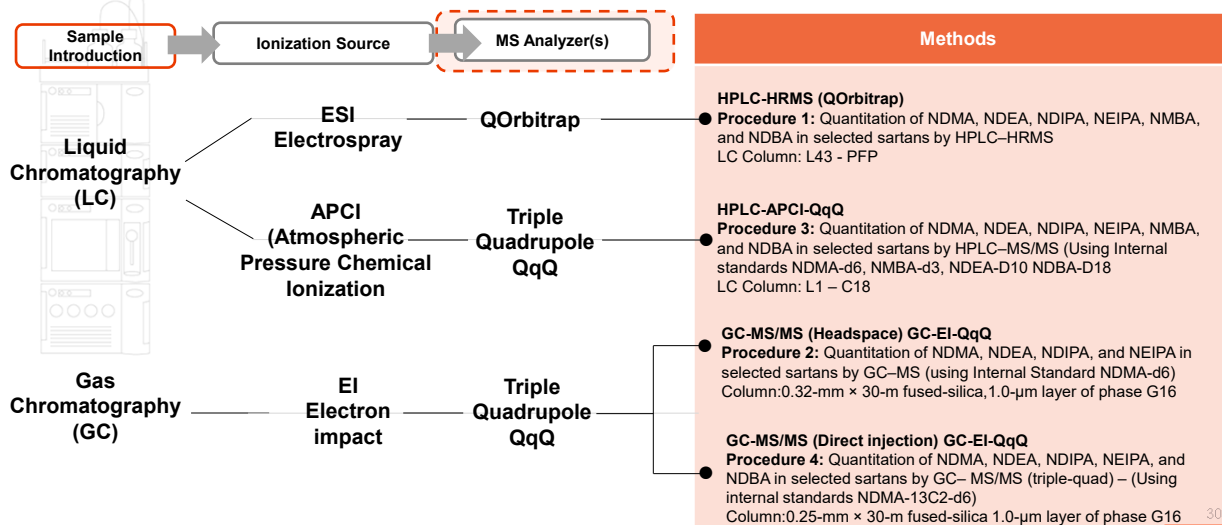
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Analytical Procedure for Nitrosamines Quantification



USP GC <1469> 8. ANALYTICAL PROCEDURES



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Analytical Procedure for Nitrosamines Quantification



General Notes

LC-HRMS (e.g.: QOrbitrap/QToF etc)

- ▶ Ranitidine DS/DP: use of LC-HRMS - avoid formation of NDMA as artifact due to ranitidine degradation
- ▶ Detecting NDMA, a volatile liquid with low molecular mass, using LC-MS can result in ion interferences and noisy chromatograms. However, High Resolution Analytical Methods offers great selectivity as the higher resolution eliminates ion interferences without the need for tandem fragmentation, making it well suited for NDMA analysis.

LC-LRMS (e.g.: APCI-QqQ)

- ▶ Ranitidine DS/DP: use of APCI-QqQ with lower temperature to avoid formation of NDMA as artifact due to ranitidine degradation
- ▶ APCI: use lower temperature in the ionization source (~250C) to prevent degradation/loss of nitrosamines

GC-MS/MS (e.g.: GC-EI-QqQ)

- ▶ Ranitidine DS/DP: High temperature applied in the inlet may generate very high levels of NDMA from ranitidine products – avoid the use of GC-MS methods for ranitidine
- ▶ The presence of dialkyl amines (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 gas chromatographic analyses.
- ▶ Total dissolution vs. selective extraction: When the API contains a dimethylamino- group, total dissolution of the DS should be avoided when applying GC techniques. High concentration of the active ingredient, when injected in the GC can generate nitrosamines in the injection port if a nitrosating agent is present: sample extractions should be modified to prevent the solubilization of the API while maintaining the extraction efficiency for nitrosamines present in the material.

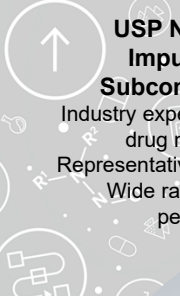
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USP Activities



General Chapter



USP Nitrosamines Impurities Joint Subcommittee (JSC)
Industry experts in impurities and drug manufacturing
Representatives from FDA/EDQM
Wide range of scientific perspectives

General Chapter


- A general chapter is better positioned as an overarching standard to address the nitrosamines impurity in several drug products and/or their components.
- Developing GC <1469> Nitrosamine Impurities – assists stakeholders (Align with current scientific and regulatory approaches)
 - General chapters numbered from 1000 to 1999 are for informational purposes only.
- Developing sub-<1000> General Chapter(s) as needed, when regulatory requirements have been finalized
 - "Applicable general chapters" means general chapters numbered below 1000 or above 2000 that are made applicable to an article through reference in General Notices, a monograph, or another applicable general chapter numbered below 1000.

General Notes

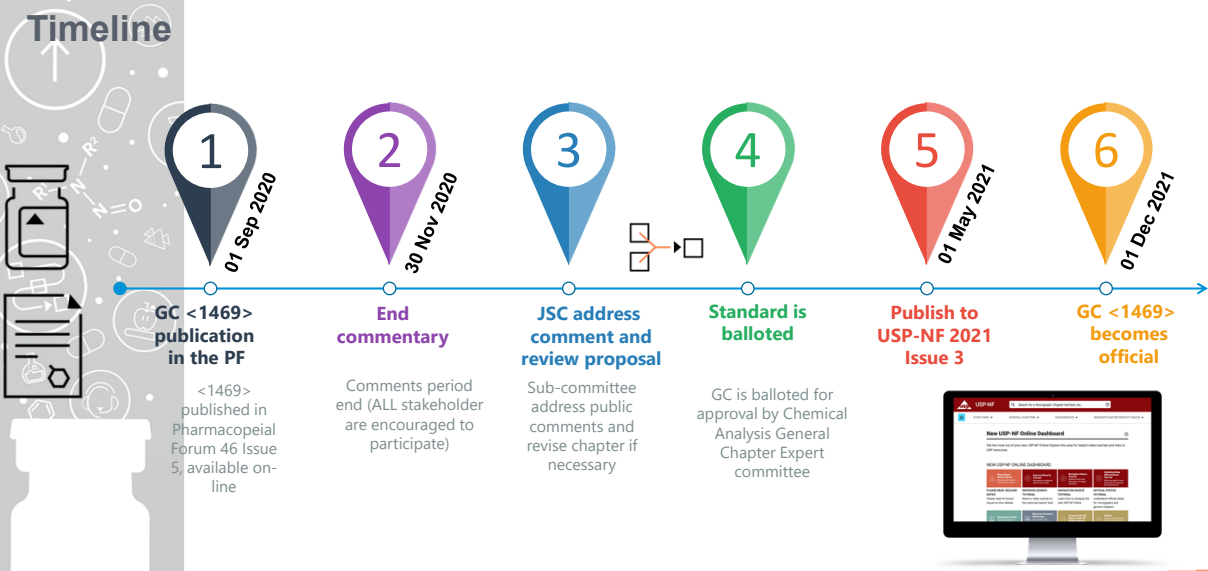
- Standards for an article recognized in the compendia (USP–NF) are expressed in the article's monograph, applicable general chapters, and General Notices.

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GC <1469> Nitrosamines Impurities



Timeline



- 01 Sep 2020**
GC <1469> publication in the PF
 <1469> published in Pharmacopeial Forum 46 Issue 5; available on-line
- 30 Nov 2020**
End commentary
 Comments period end (ALL stakeholder are encouraged to participate)
- JSC address comment and review proposal**
 Sub-committee address public comments and revise chapter if necessary
- Standard is balloted**
 GC is balloted for approval by Chemical Analysis General Chapter Expert committee
- 01 May 2021**
Publish to USP-NF 2021 Issue 3
- 01 Dec 2021**
GC <1469> becomes official

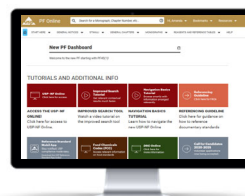
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GC <1469> Nitrosamines Impurities



Content and Rationale

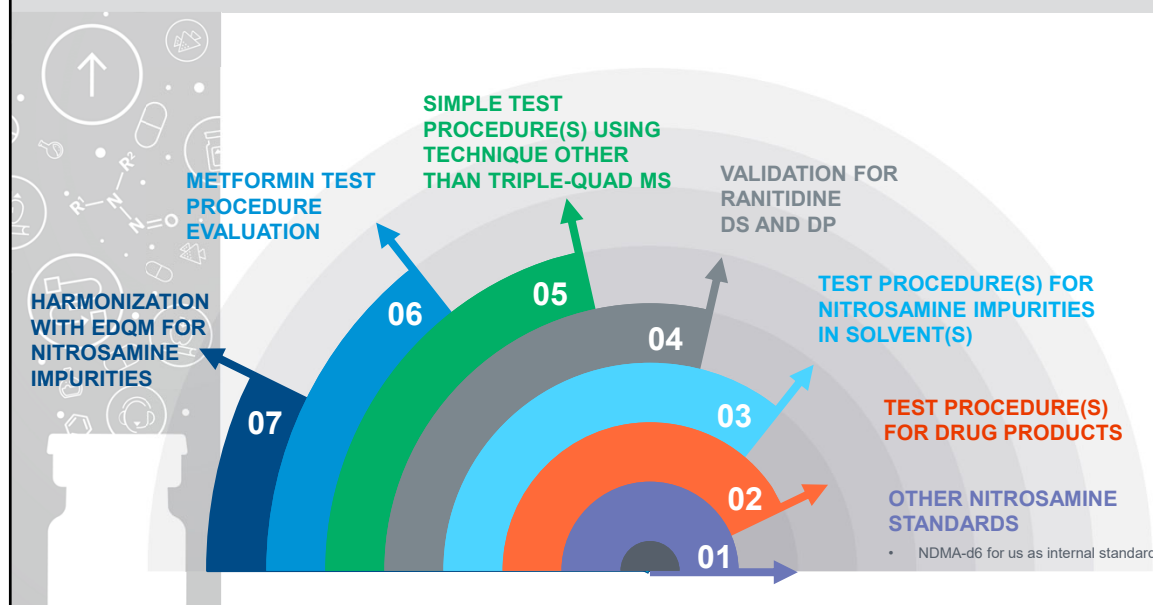
1. INTRODUCTION
2. NITROSAMINE IMPURITIES
3. SOURCES OF NITROSAMINES
4. NITROSAMINE RISK ASSESSMENTS – DEVELOPMENT OF A CONTROL STRATEGY
5. LIMITS OF NITROSAMINE
6. TESTING FOR THE PRESENCE OF NITROSAMINES
7. TEST METHOD PERFORMANCE CHARACTERISTICS OF NITROSAMINE METHODS
8. ANALYTICAL PROCEDURES (Quantitative Analytical Procedures)
9. ADDITIONAL SOURCES OF INFORMATION



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USP Activities - On-going development



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Reference Standards



Newly released USP Reference Standards for comprehensive analysis for nitrosamines in pharmaceuticals

- Trace level quantification using LC/MS, GC/MS or other suitable techniques
- Useful for various test methods (FDA, in house, etc.)

Released on June 2020

- N-Nitrosodimethylamine (NDMA)
(1 mg/mL in methanol)
- N-Nitrosodiethylamine (NDEA)
(1 mg/mL in methanol)
- N-Nitrosodiisopropylamine (NDIPA)
(1 mg/mL in methanol)
- N-Nitrosodibutylamine (NDBA)
(1 mg/mL in methanol)
- N-Nitrosoethylisopropylamine (NEIPA)
(1 mg/mL in methanol)
- N-Nitrosomethylaminobutyric acid (NMBA)
(1 mg/mL in acetonitrile)



Knowledge Hub



Protecting patients from harmful impurities

Home / Our Work / Chemical Medicines

Nitrosamine impurities

To protect patients and strengthen the global medicines supply chain, USP is providing tools and solutions to analyze and monitor emerging impurities in the drug supply.

Starting in 2018 the presence of nitrosamine impurities was identified in some angiotensin II receptor blocker (ARBs) used to treat high blood pressure and heart failure. Subsequently, nitrosamines impurities have been found in additional drug products, leading to a major effort by regulators and industry to reduce or eliminate their presence in the drug supply.

Companies are responsible for understanding their manufacturing processes, which includes identifying and preventing the presence of unacceptable impurities. This involves developing new predictive approaches, along with using suitable methods to detect and control these impurities as well as others that may arise when making changes to manufacturing processes.

USP is supporting manufacturers and regulators with tools and solutions for testing, assessing risk and understanding potential sources related to nitrosamine impurities.

For additional information or questions about USP's efforts related to nitrosamine impurities, contact nitroch@usp.org.

<https://www.usp.org/chemical-medicines/nitrosamine-impurities>

Genotoxic impurities webinar

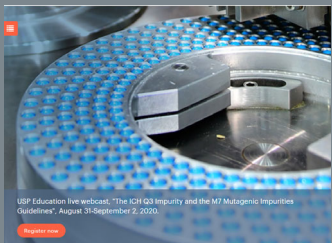
Ed Gupta, PhD, Vice President, USP Small Molecules Pharmaceutical Technology Editors Series Webcast
"Genotoxic Impurities and Drug Quality: Lessons from the Nitrosamine Contamination Crisis"
July 14, 2020

[View the webinar recording](#)

Methods for detecting & controlling nitrosamines

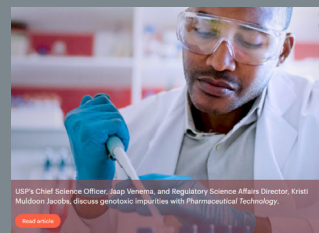
USP has announced the development of an informational chapter, including proposed methods, for publication in the United States Pharmacopeia–National Formulary (USP–NF).

[View chapter](#)



USP Education live webcast, "The ICH Q3 Impurity and the M7 Mutagenic Impurities Guidelines", August 21-September 22, 2020.

[Register now](#)



USP's Chief Science Officer, Jasp Verma, and Regulatory Science Affairs Director, Kristi Muldown-Jacobs, discuss genotoxic impurities with Pharmaceutical Technology.

[Read article](#)

Act NOW!



- 1) Access USP-PF
- 2) Download <1469> Nitrosamine Impurities
- 3) Submit your comments (11-Nov-20)
- 4) Register for Webinar 2
<1469> Nitrosamine Impurities – Deep Dive
- 5) Reach out to your SCD Manager.
Stay up-to-date



https://online.usppf.com/usppf/document/GUID-C97F817C-A383-4693-8E0C-2F0A0A371977_10101_en-US

Thank You

