

The Chemistry of N-Nitrosamines: An Overview

Steven W. Baertschi, Ph.D.

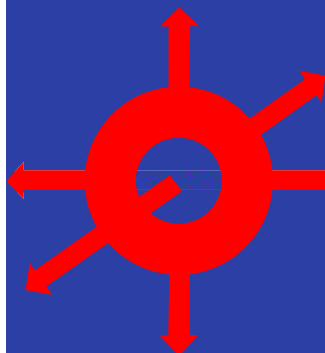
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Oct 31, 2023

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Outline

Disclaimer:

**This is an overview:
not a comprehensive
chemistry review!!**

➤ Introduction

- Historical context

➤ Chemistry

- Nitrosating reagents / N-Nitrosation reactions
- Rates
- N-Nitrosation Examples

➤ Assessing the Risk

- Is there a risk?
- What levels can we predict?

➤ Potential for Inhibition

➤ Stability of N-Nitrosamines

➤ Conclusions

1970s-80s: “Nitrosatability” of Amines and Amine-Containing Drugs

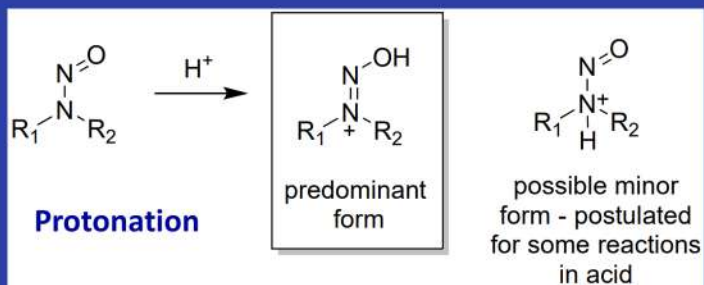
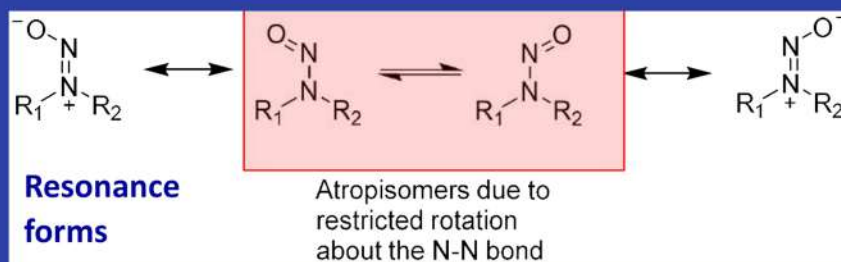
Significant concern in 1970s and 1980s over the acid-catalyzed reaction of nitrites and nitrates with certain amines

- Concern focused presence in foods and formation **in vivo**
- **1978:** WHO Nitrosation Assay Procedure (NAP Test)¹ developed
 - ✓ Conditions were developed to rank drugs re: nitrosatability under “standardized”² as well as under “simulated gastric”³ conditions
 - ✓ Dramatic variability (>30,000-fold differences in rates)
 - ✓ Illustrated potential for N-nitrosation of drugs in vivo from oral administration



1. Coulston F (1980), in “The potential carcinogenicity of nitrosatable drugs”, Coulston F, Dunne JF, Eds, Proceedings of a WHO Symposium, Geneva, June (1978), p. 8, Albex Publishing Co, Norwood, NJ USA.
2. Gillat PN, Hart RJ, Walters CL, “Susceptibilities of drugs to nitrosation under standardized chemical conditions”, Fd. Chem. Toxic., 22:4, 269-274 (1984).
3. Gillat PN, Palmer RC, Smith PLR, Walters CL, “Susceptibilities of drugs to nitrosation under simulated gastric conditions”, Fd. Chem. Toxic., 23:9, 849-855 (1985).

Resonance Forms, Protonation, and Denitrosation



Scheme 2. Proposed Mechanism for Nitrosamine Denitrosation in Acid via the N-Protonated Conjugate Acid (5)



Pathways for N-nitroso compound formation: secondary amines and beyond

- Lopez-Rodriguez R et al., Org. Proc. Res. Dev., 24, 1558-1585 (2020)

An organic chemist's guide to N-nitrosamines

- J. Org. Chem. 2021, 86, 2037–2057

Protolytic denitrosation – accelerated by nucleophiles such as bromide, thiocyanate, and thiourea to the reaction

N-Nitrosamine Formation: Primary Concerns - Chemical Synthesis

- **Primary concerns during synthesis of the API appear to be related to exposure of secondary or tertiary amines to nitrites (used during aqueous workups)** - “Lessons learnt from presence of N-nitrosamine impurities in sartan medicines: Overview and recommendations”, 23 June 2020, EMA/526934/2019

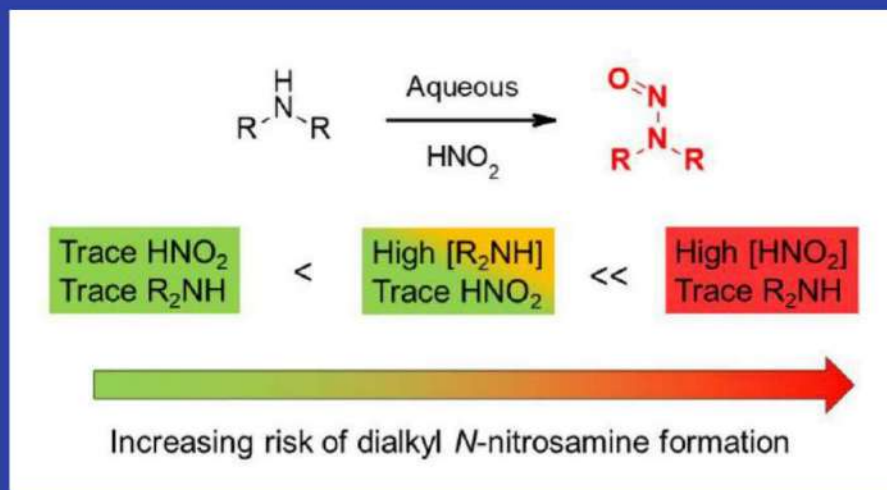


Figure from Ashworth IW, Dirat O, Teasdale A, Whiting WP, “Potential for the formation of Nnitrosamines during the manufacture of APIs: an assessment of the risk posed by trace nitrite in water”, Org. Process Res. Dev. 24:9, 1629-1646 (2020)

More Recently: Formation in the Drug Product

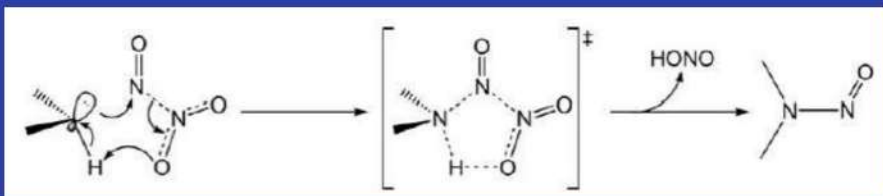
- Concerns about present / formation of N-nitrosamines in formulated drug products from API degradation
 - “Nitrates and nitrites can be found in many excipients at ppm levels.”¹
 - ✓ “The exact source of these trace impurities has not been investigated, but it is possible that they could come from process water, processing steps requiring acid titration, bleaching, and potentially from oxidation in air during high temperature drying.”¹
- Lhasa Ltd has compiled an excipient nitrite database²

1. Wu et al., AAPS PharmSciTech, 12:4, 1248-1263, Dec (2011).

2. Boetzel R, J. Pharm. Sci. Jun;112(6):1615-162 (2023)

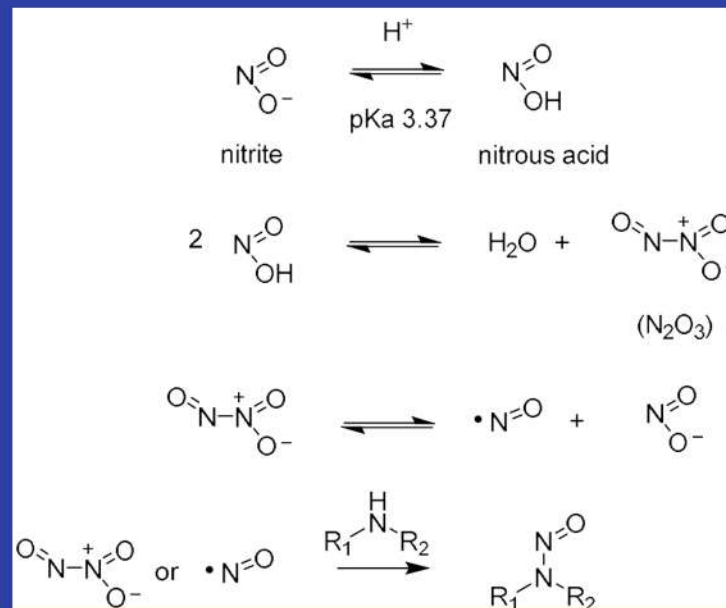
Nitrosating Reagents Relevant to Drug Product Formulations

➤ Nitrites



J. Mol. Model. 17:669-680 (2011).

No longer true – there are now many nitrite examples!

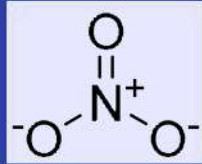


There have been ~~no reports of the formation of N-nitrosamines in drug products attributed to nitrates and nitrites in excipients.~~ However, this possibility is considered a potential root cause and cannot yet be ruled out.¹

1. "Lessons learnt from presence of N-nitrosamine impurities in sartan medicines: Overview and recommendations", 23 June 2020, EMA/526934/2019

Nitrosating Reagents Relevant to Drug Product Formulations

➤ ~~Nitrates~~

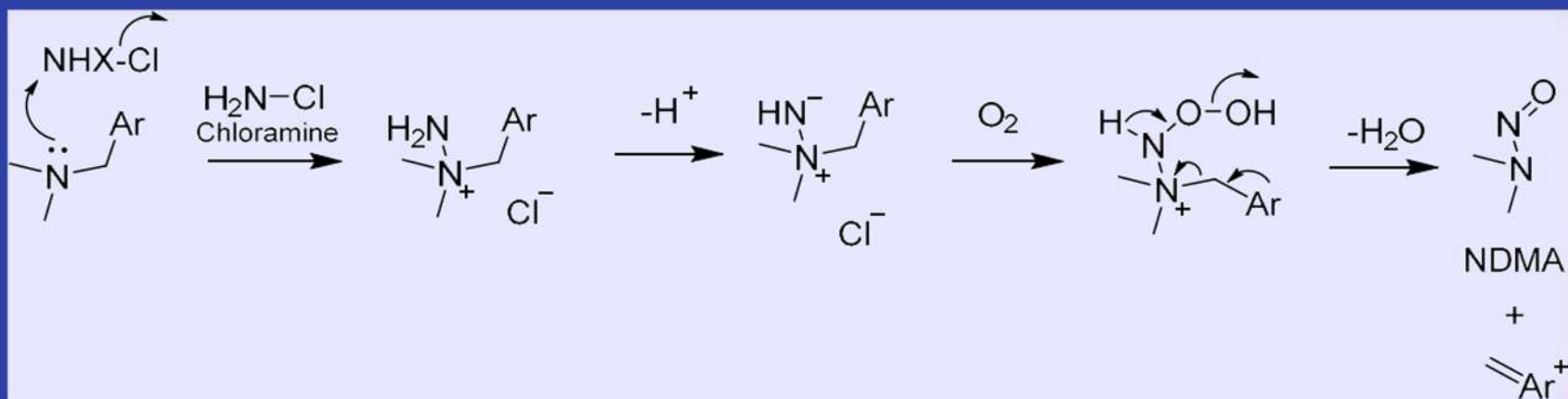


- ✓ Nitrates do not N-nitrosate directly – must first be reduced to nitrite. **Can occur *in vivo***, but not known to occur in drug products
- ✓ Nitrates can decompose to nitrites **at very high temperatures (e.g., >100-1000C)** but this does not appear to be relevant to drug product storage / distribution
 - ✓ May be relevant to vulcanization processes / elastomer formation processes*

*Boltres B, "Evaluating nitrosamines from elastomers in pharmaceutical primary packaging, PDA Journal of Science and Technology, July 2021, pdajpst.2021.012645; DOI: <https://doi.org/10.5731/pdajpst.2021.012645>

Nitrosating Reagents Relevant to Drug Product Formulations

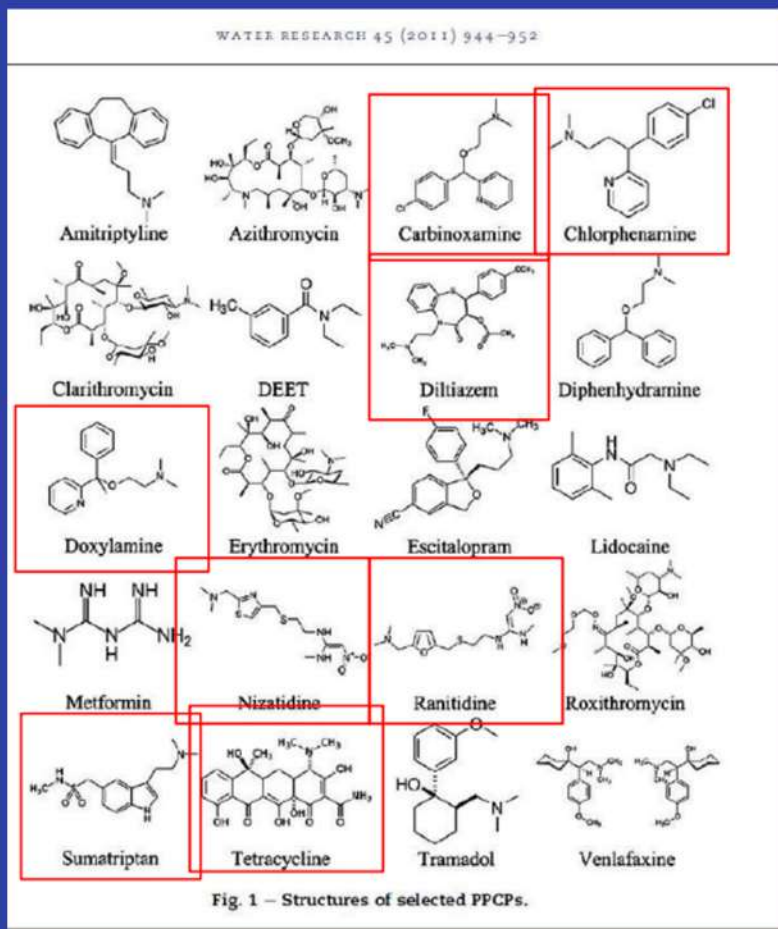
➤ **Chloramines:** from water disinfection processes



1. LeRoux J, Gallard H, Croue J-P, Papot S, Deborde M, "NDMA formation by chloramination of ranitidine: kinetics and mechanism", *Environ. Sci. Technol.* 46, 11095-11103 (2012).
2. Liu YD, Selves M, Zeng C, Zhong R, Karanifil T, "Formation mechanism of NDMA from ranitidine, trimethylamine, and other tertiary amines during chloramination: a computational study", *Environ. Sci. Technol.* 48, 8653-8663 (2014).

Nitrosating Reagents Relevant to Drug Product Formulations

➤ **Chloramines:** from water disinfection processes



- ✓ Susceptibility of 20 APIs to nitrosamine formation after exposure to water disinfected with chloramine
- ✓ Results showed the potential for nitrosamine formation, especially for 8 of the APIs.
- ✓ Focus was on **wastewater** treatment – high levels of chloramines, 24 hr, 21C, pH 7.
- ✓ **No examples from API or DP manufacturing processes**

Shen R, Andrews SA, Water Research, 45, 944-952 (2011)

Unexpected Source of Nitrites

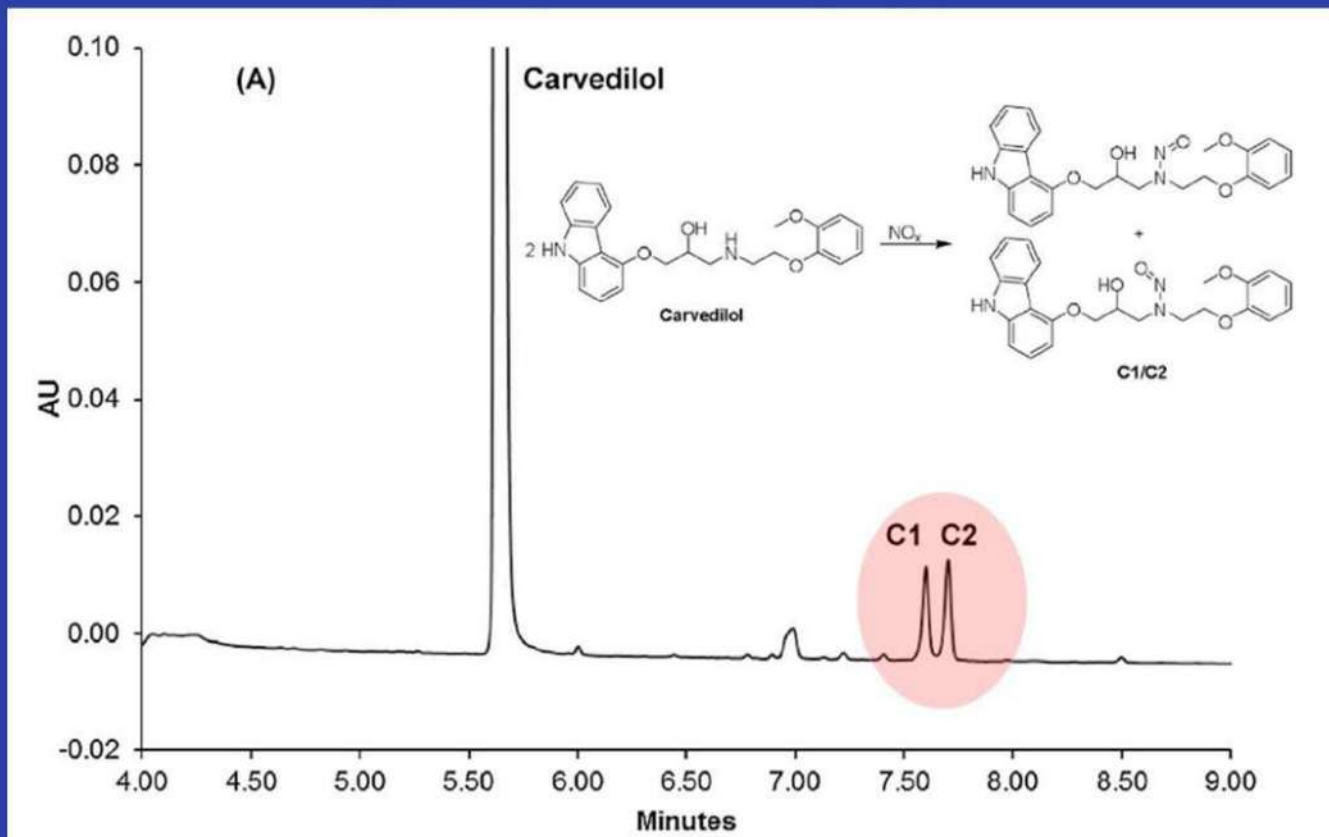
- **Nitrite leaching from certain glass HPLC vials***
- **Created nitrite esters** of the API (clofazimine), observable after 24 h, continued growth up to ~2%
- About **10-15% of the vials** at RT showed this leachable-related degradation pathway when stored at RT
- Can **suppress by storing at 2-8C or in less acidic diluents**



*Ghost peak of clofazimine: a solution degradation product of clofazimine via nucleophilic substitution by nitrite leaching from certain glass HPLC vials, Lin J et al., JPBA, 150, 183-190 (2018)

Potentially Relevant “Artifactual” N-Nitrosation

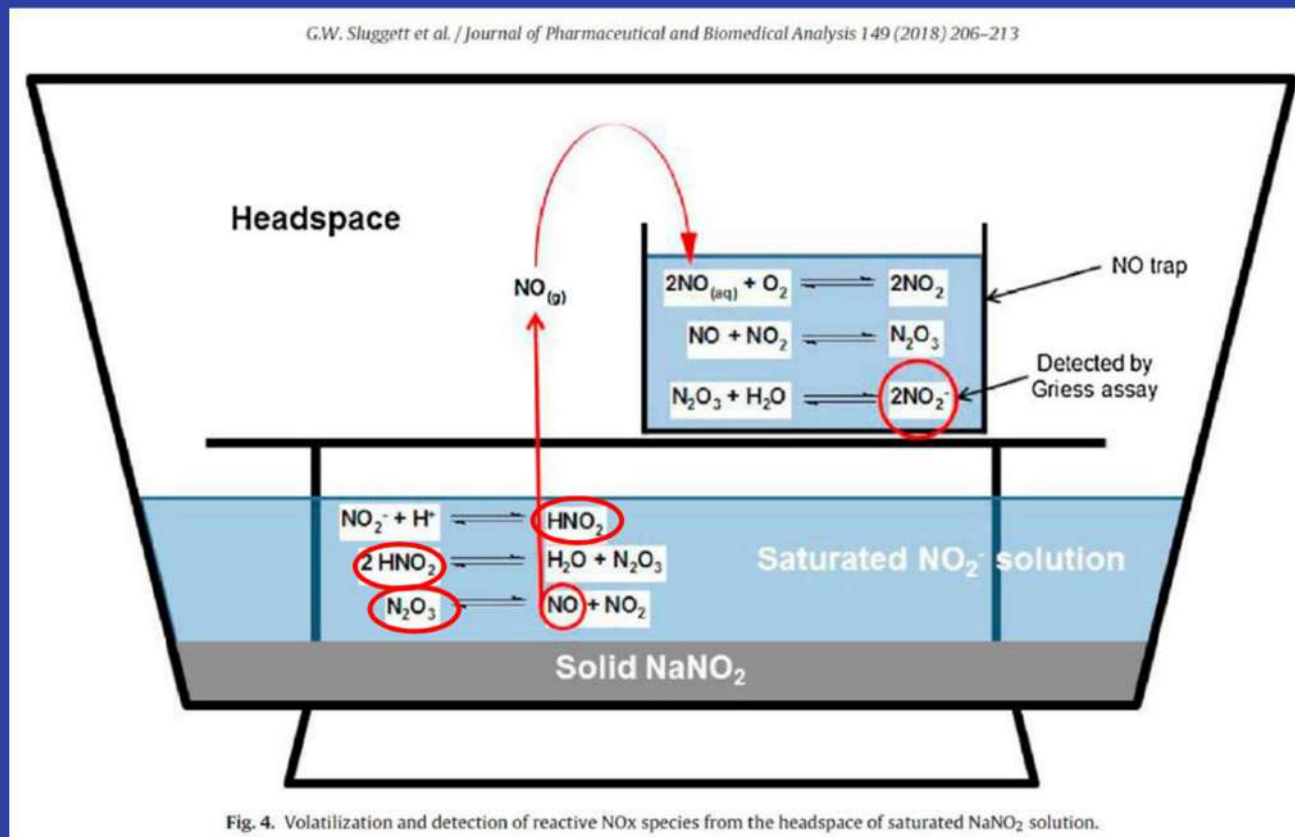
- Saturated salts for humidity control: NaNO_2 for 60% RH



Sluggett GW, Zelesky T, Hetrick EM, Babayan Y, Baertschi SW, “Artifactual degradation of secondary amine containing drugs during accelerated stability testing when saturated sodium nitrite solutions are used for humidity control”, *J. Pharm. Biomed. Anal.*, 149, 206-213 (2018).

Potentially Relevant “Artifactual” N-Nitrosation

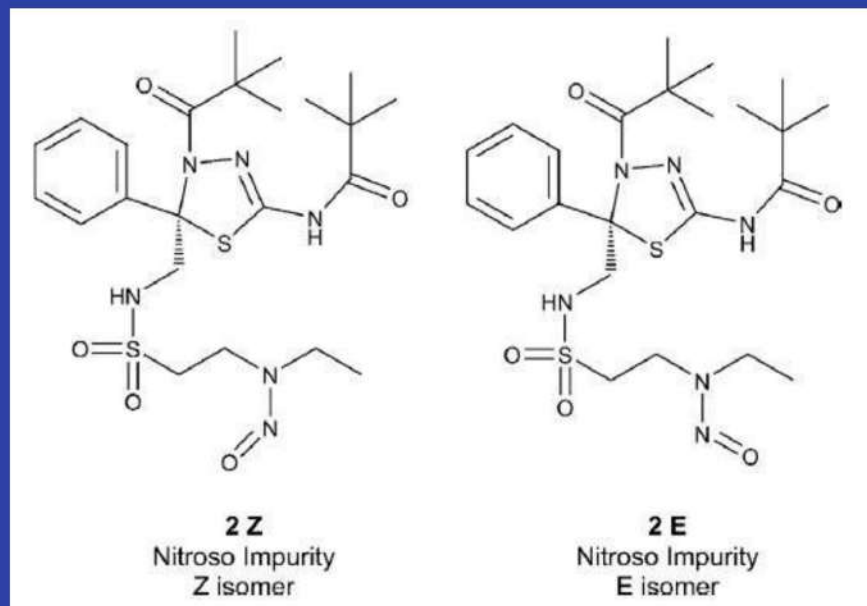
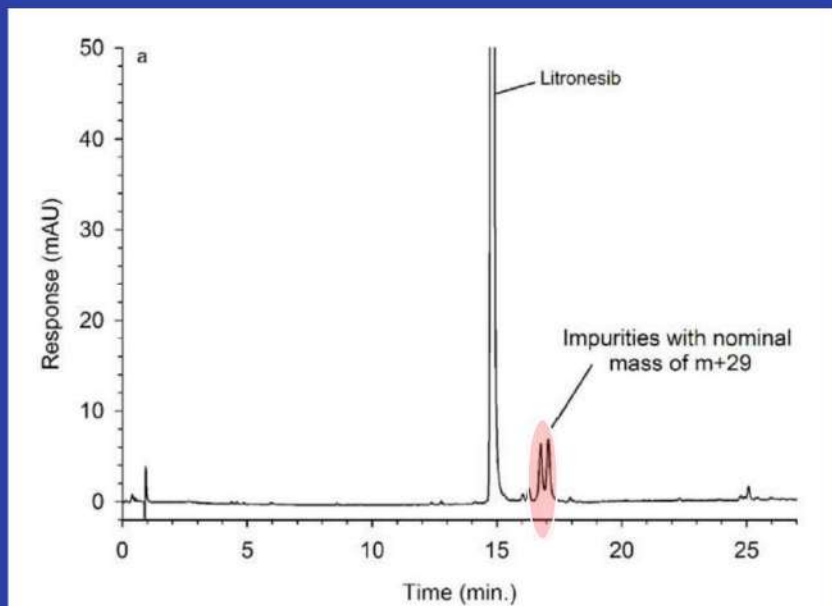
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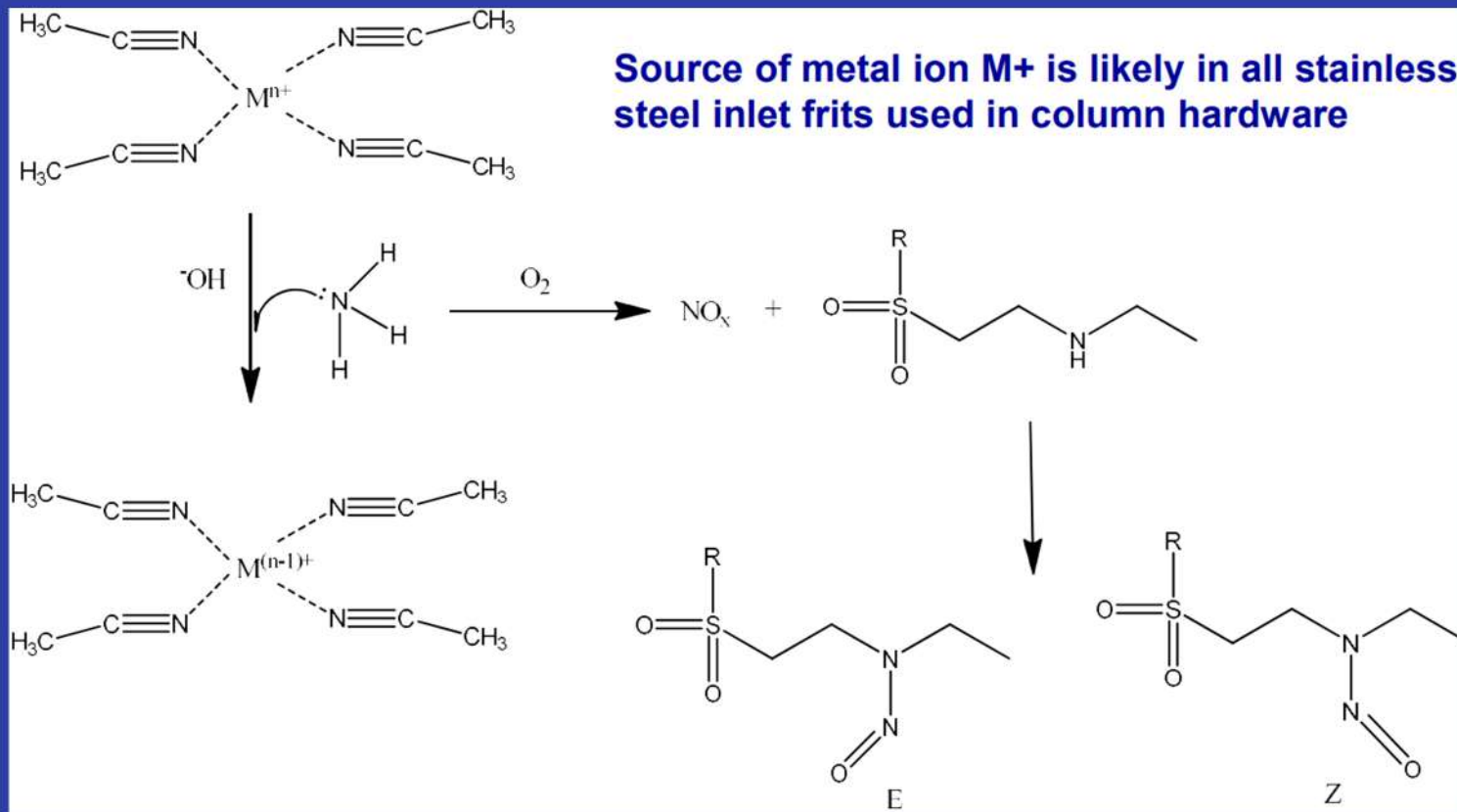
Potentially Relevant “Artifactual” N-Nitrosation

- Unexpected on-column (HPLC) N-nitrosation reaction
- Requires ammonium hydroxide, high pH, stainless steel frits, and acetonitrile as the organic modifier,



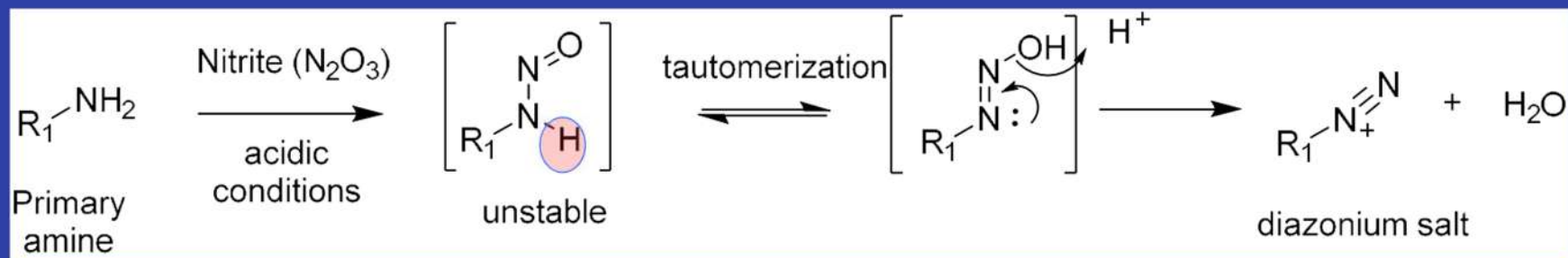
Myers DP et al., “On column nitrosation of amines observed in liquid chromatography impurity separations employing ammonium hydroxide and acetonitrile as mobile phase”, *J. Chrom. A.*, 1319, 57-64 (2013).

Current Hypothesis for Mechanism of Nitrosamine formation



Primary Amines

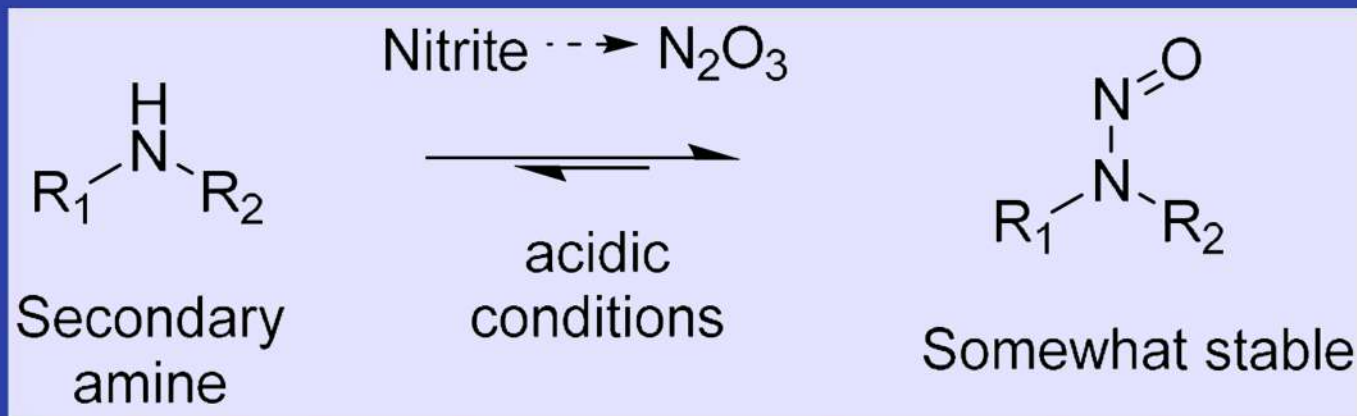
- "Primary amines react readily with nitrite, but the presence of adjacent hydrogens allows rapid conversion of the nitrosamine species to a diazonium salt. Therefore, formation of N-nitrosamine impurities is not considered a major risk when only primary amines are present"*



*"Lesson learnt from presence of N-nitrosamine impurities in sartan medicines: Overview and recommendations", 23 June 2020, EMA/526934/2019

Secondary Amines

- **“Secondary Amines”** For secondary amines, the pathway to diazonium species is blocked by the presence of adjacent alkyl groups. Secondary amines and their ammonium salts react readily with nitrite to form the associated nitrosamine species...^{***}

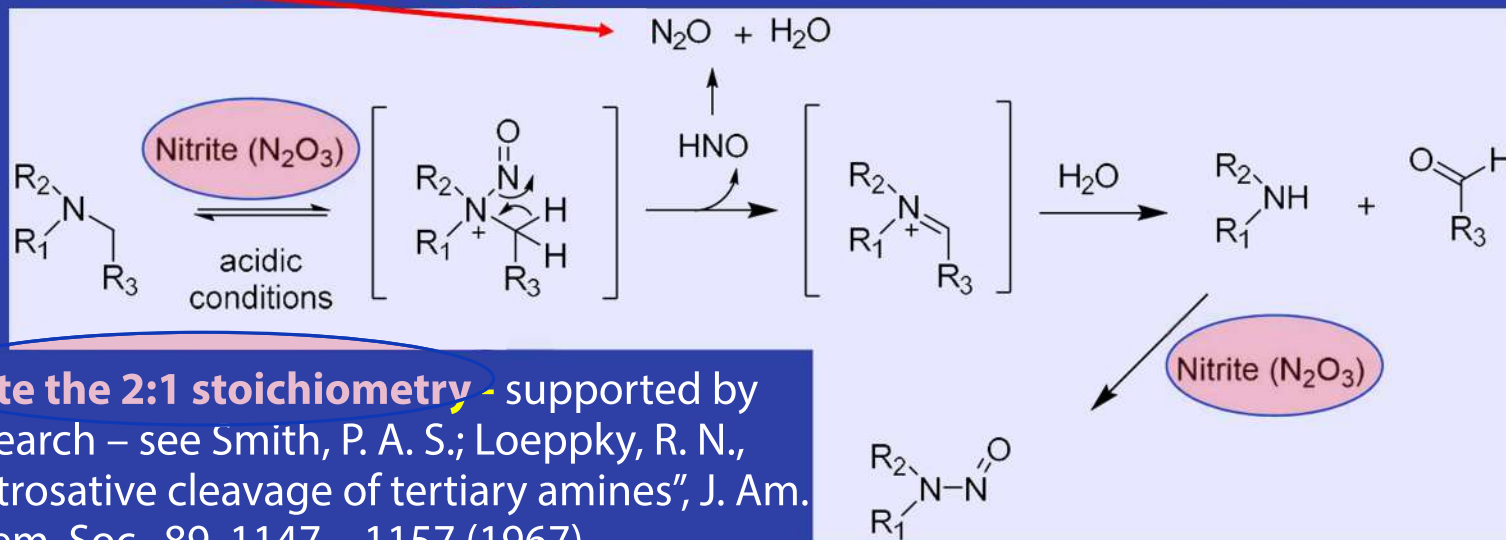


^{***}“Lesson learnt from presence of N-nitrosamine impurities in sartan medicines: Overview and recommendations”, 23 June 2020, EMA/526934/2019

Tertiary Amines / Quaternary Salts

- **“Tertiary Amines”** Tertiary amines and their ammonium salts are also known to react directly with nitrites to form N-nitrosamines via a dealkalytive mechanism...quaternary ammonium salts are able to form N-nitrosamines almost at the same level by a similar dealkaylation reaction.”*

Nitrous oxide – does not cause nitrosation



Note the 2:1 stoichiometry - supported by research – see Smith, P. A. S.; Loepky, R. N., “Nitrosative cleavage of tertiary amines”, J. Am. Chem. Soc., 89, 1147 – 1157 (1967).

*“Lesson learnt from presence of N-nitrosamine impurities in sartan medicines: Overview and recommendations”, 23 June 2020, EMA/526934/2019

N-Nitrosation Rates

- **Acidic conditions** : favored for formation of the nitrosating species (N_2O_3) from nitrite
- **Basic conditions**: favored for reaction of the amine with the nitrosating species
- The N-nitrosation rate is greatest:
 - For secondary and tertiary amines, at **pH ~2.5-3.4**
 - **The higher the pKa, the slower the N-nitrosation reaction**
 - For secondary amides, the rate at pH 1 is > pH 3
- Tertiary amine N-nitrosation rates are, in general, >1000x slower (~100- 10,000x) than their analogous secondary amines!!
 - N-nitrosation only occurs if there are protons alpha to the nitrogen
 - Mitch W, "Literature review of nitrosamine and nitramine formation chemistry relevant to carbon capture" (2019)
 - Mirvish SS, "Formation of N-nitroso compounds: chemistry, kinetics, and in vivo occurrence", Toxicol. Appl. Pharmacol. 31, 325-351 (1975).

N-Nitrosation Rates

Fastest rate of N-nitrosation for secondary amines (pH 2.5-3.4)

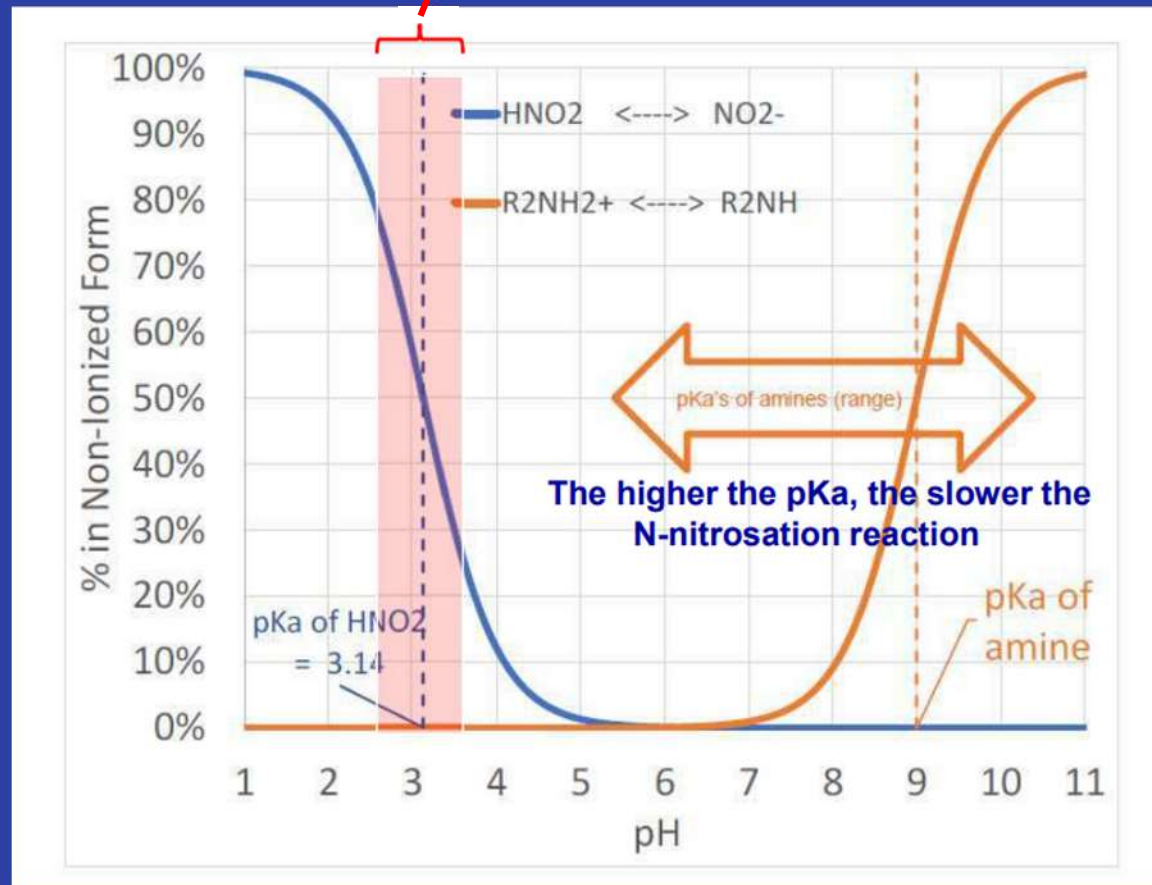
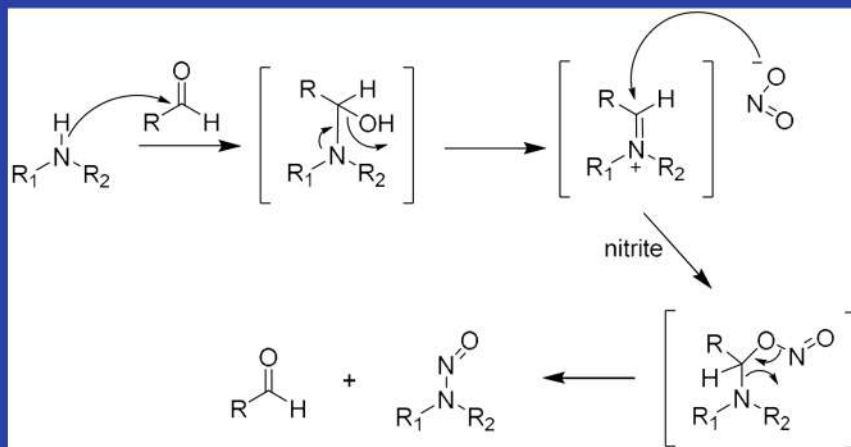


Figure adapted from G. Scrivens (Pfizer), presented at Science of Stability 2021

N-Nitrosation at Neutral to Basic pH

- Small aldehydes such as formaldehyde or chloral (**at very high levels in solution**) can increase the rate of N-nitrosation reaction at higher pHs¹
- Harmon has proposed this **may be relevant to Solid State formulations**²



¹Keeper LK, Roller PP, "N-Nitrosation by nitrite ion in neutral and basic medium", Science, vol 181 (4106), 1245-1247 (1973)

²Harmon P, Trace aldehydes in solid oral dosage forms as catalysts for nitrosating secondary amines, J. Pharm. Sci., 112:5, 1216-1219 (2023)

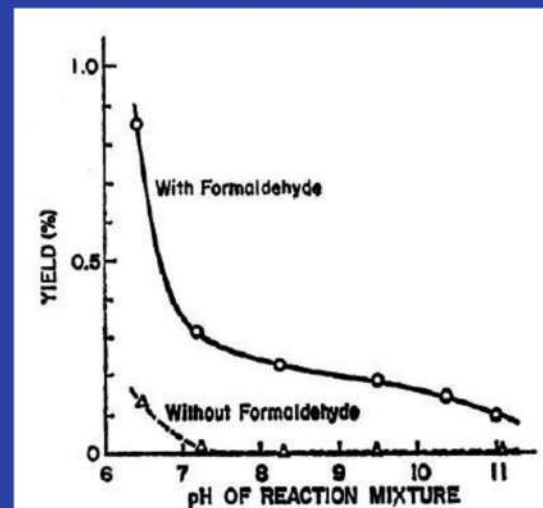
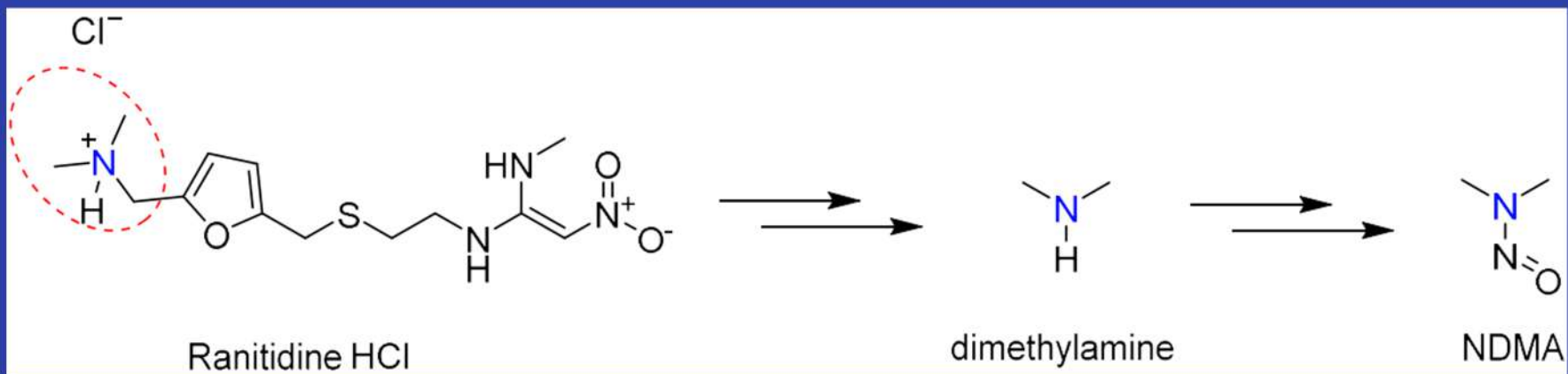


Fig. 1. Percent yield of diethylnitrosamine after 17 hours at 24°C as a function of pH in the reaction of 0.05M diethylamine with 0.2M sodium nitrite (in 0.5M phosphate buffer with and without 0.05M formaldehyde).

Assessing the Risk: Ranitidine as an Example



Ranitidine: Mechanism of NDMA formation

Ranitidine—Investigations into the Root Cause for the Presence of *N*-Nitroso-*N,N*-dimethylamine in Ranitidine Hydrochloride Drug Substances and Associated Drug Products

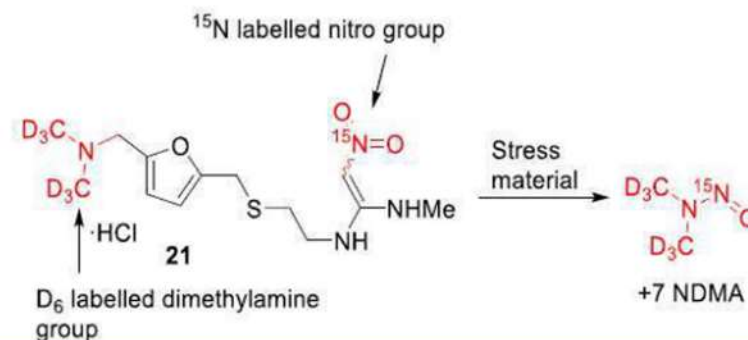
Fiona J. King, Andrew D. Searle,* and Michael W. Urquhart

Org. Process Res. Dev. 2020, 24, 2915–2926

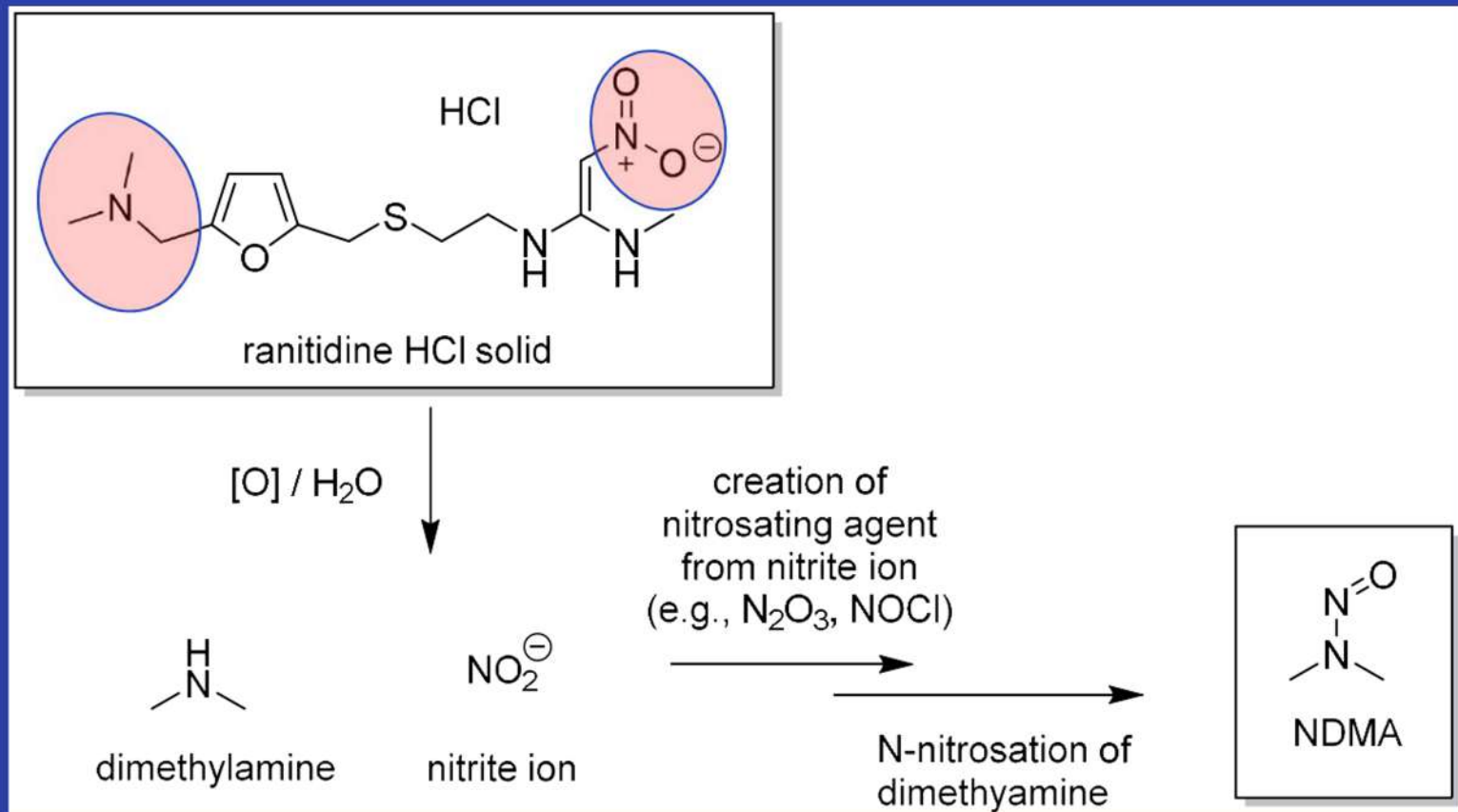
ABSTRACT: The presence of low levels of *N*-nitroso-*N,N*-dimethylamine (NDMA) in ranitidine hydrochloride drug products has been reported by regulatory agencies. GlaxoSmithKline undertook a root cause analysis to investigate this observation using contemporaneous, highly sensitive analytical methodologies. The root cause analysis suggested that the presence of NDMA results from a slow degradation of the ranitidine molecule. Analysis using suitably isotopically labeled ranitidine hydrochloride confirmed the formation of NDMA solely from an intermolecular reaction of ranitidine hydrochloride without involvement of impurities. Factors that influence the rate of degradation include heat, humidity, and the crystal morphology of ranitidine hydrochloride with the material exhibiting a columnar habit showing a slower rate of degradation.

KEYWORDS: ranitidine hydrochloride, *N*-nitroso-*N,N*-dimethylamine (NDMA), degradation

Scheme 2. Proposed Formation of +7 NDMA from Isotopically Labeled Ranitidine Hydrochloride

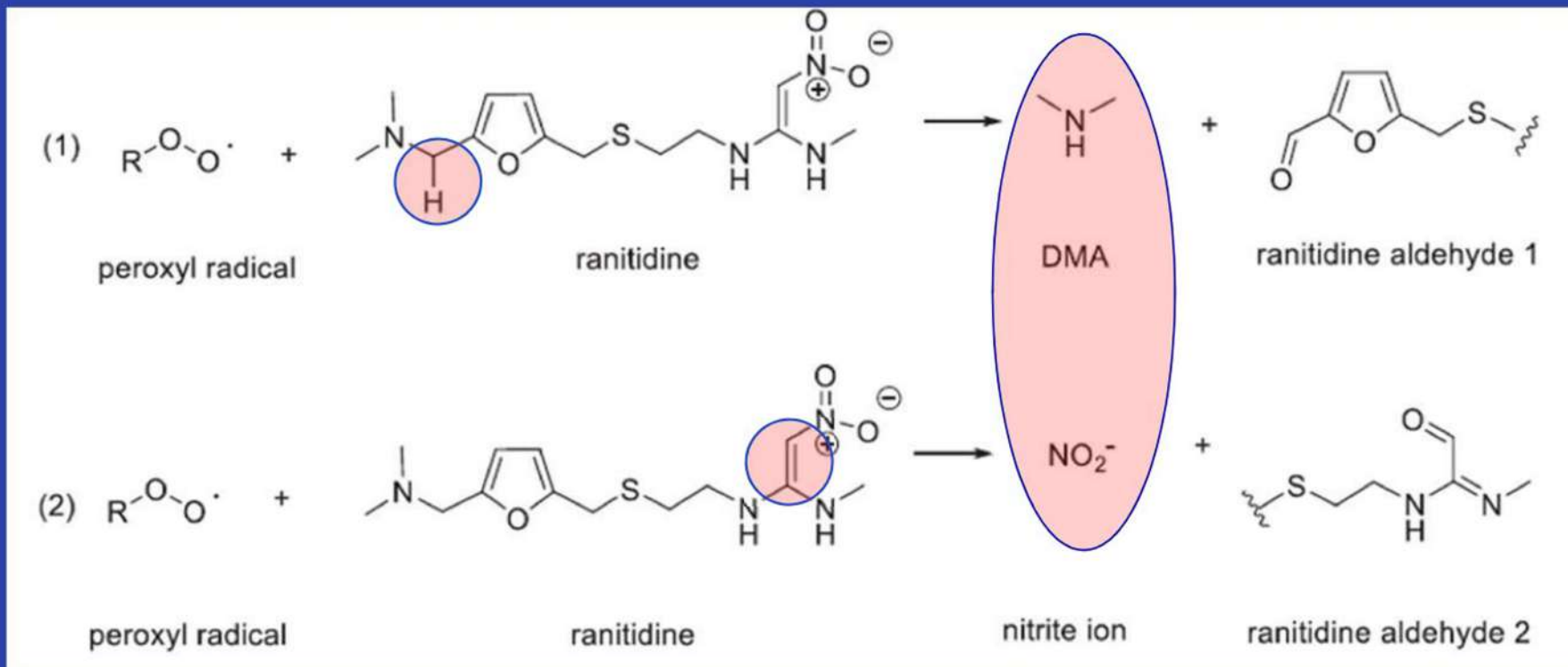


Ranitidine: Mechanism of NDMA formation



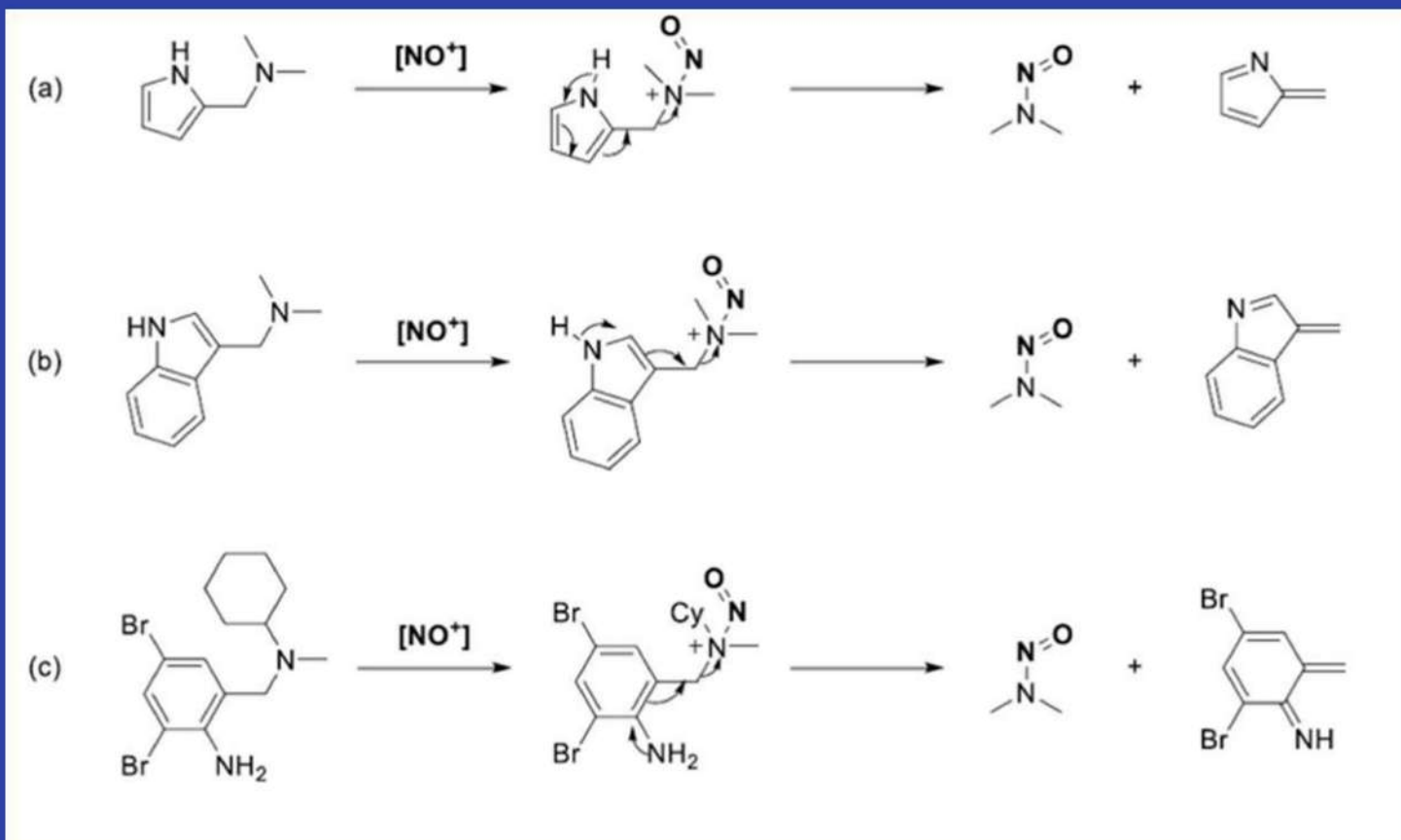
Ranitidine: a proposed mechanistic rationale for NDMA formation and a potential control strategy, Harmon P, J. Pharm. Sci., 112(5):1220-1224 (2023)

Ranitidine: Proposed Mechanism of NDMA Formation



Ranitidine: a proposed mechanistic rationale for NDMA formation and a potential control strategy, Harmon P, *J. Pharm. Sci.*, 112(5):1220-1224 (2023)

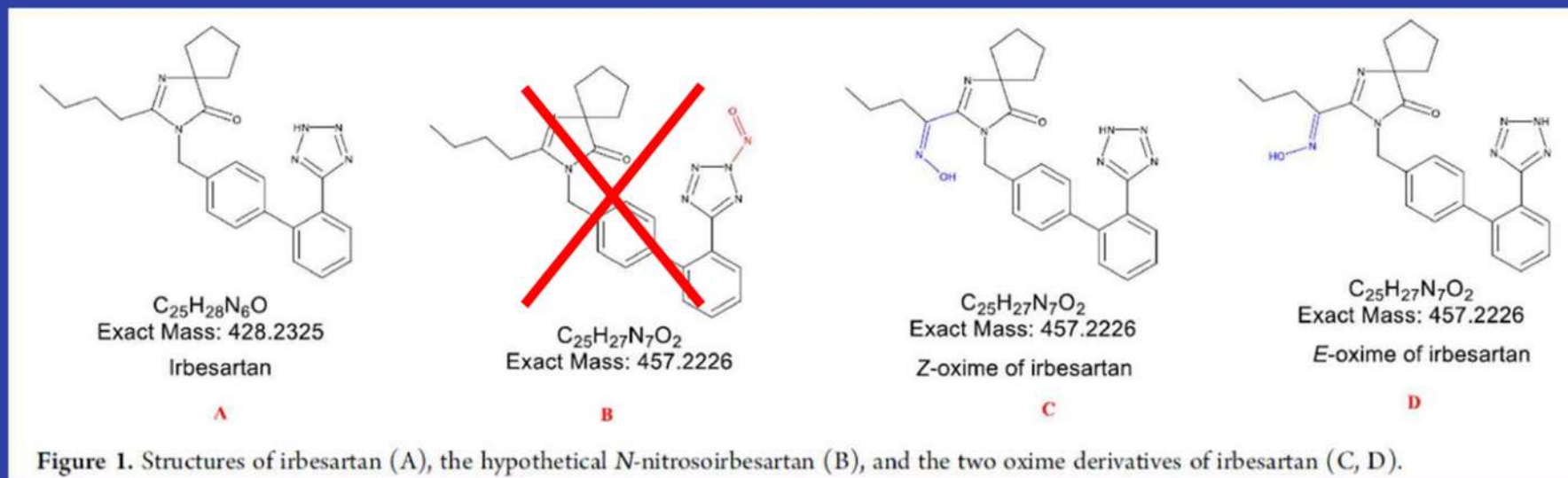
Rapid Nitrosative Dealkylation of Certain Aromatic Systems



Pathways for N-nitroso compound formation: secondary amines and beyond, LopezRodriguez R et al., *Org. Proc. Res. Dev.*, 24, 1558-1585 (2020)

N-Nitroso-irbesartan??

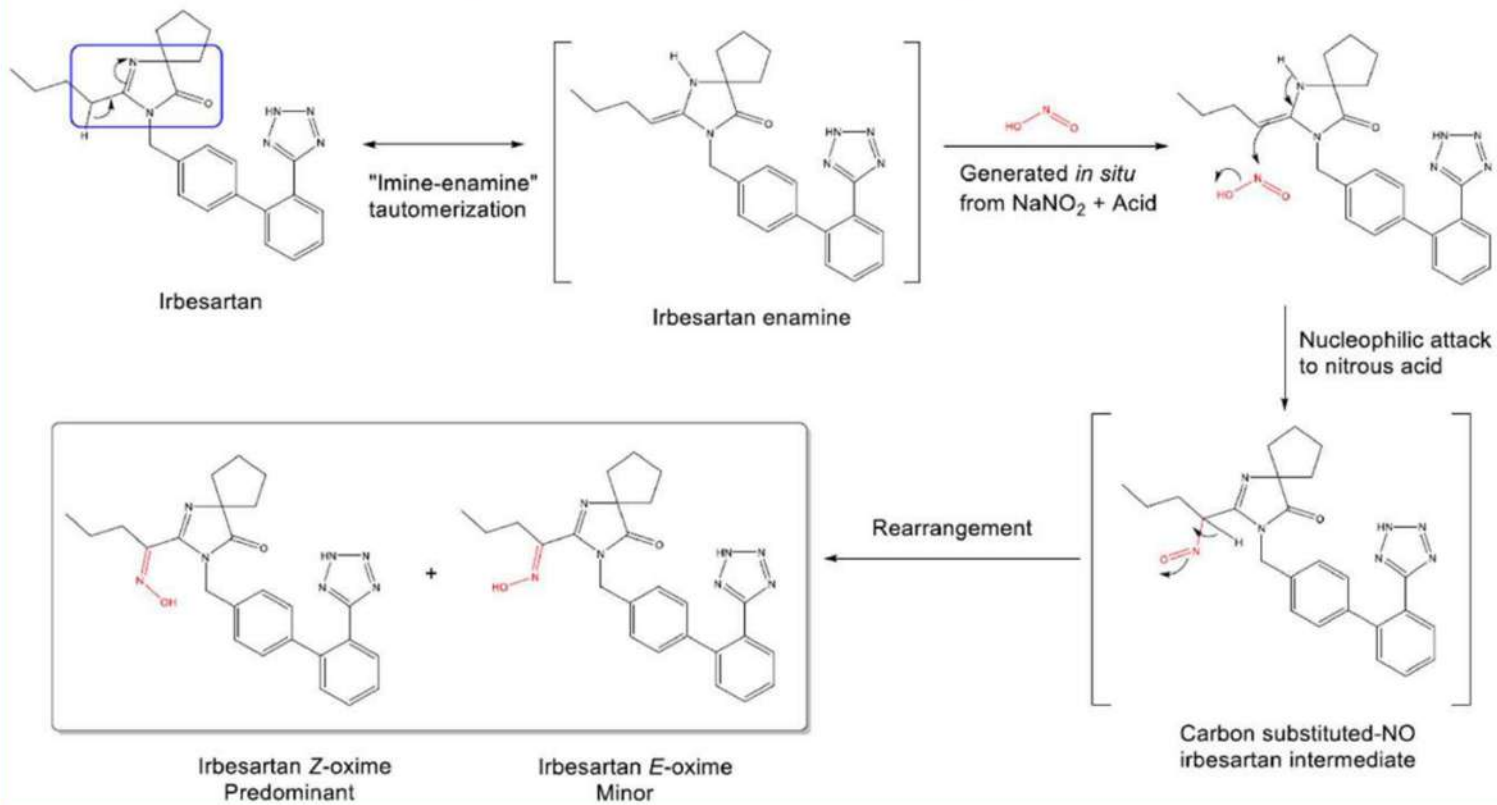
- Oct 2021: Irbesartan / irbesartan hydrochlorothiazide finished products recalled on US market – **presence of N-nitroso-irbesartan impurity?**
- Reactions with nitrous acid showed this impurity to be **isomeric oxime derivatives!!**



- Reaction of irbesartan with nitrous acid produces irbesartan oxime derivatives, rather than N-nitrosoirbesartan, OPRD, 26, 1236-1246 (2022)

Formation of E- and Z-Oximes of Irbesartan

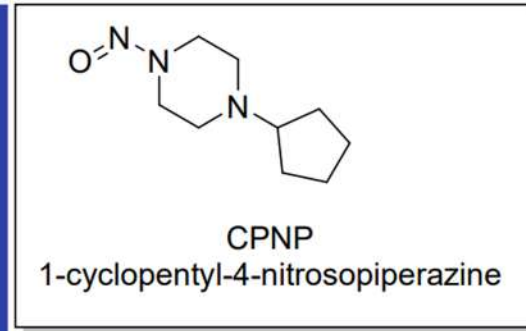
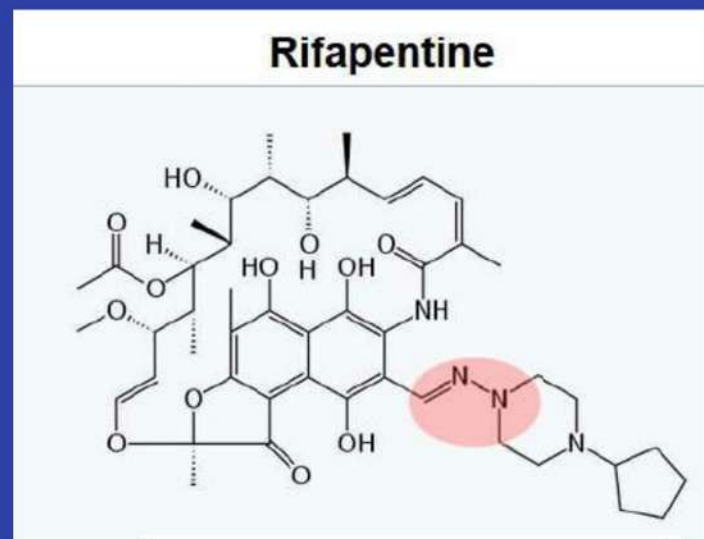
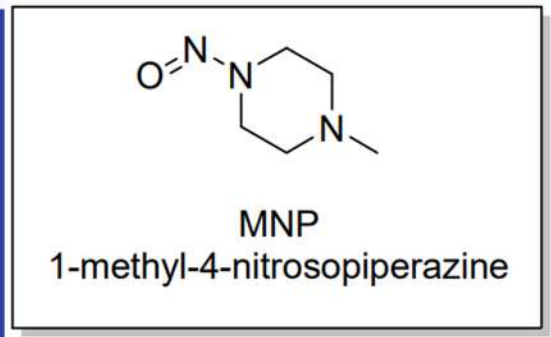
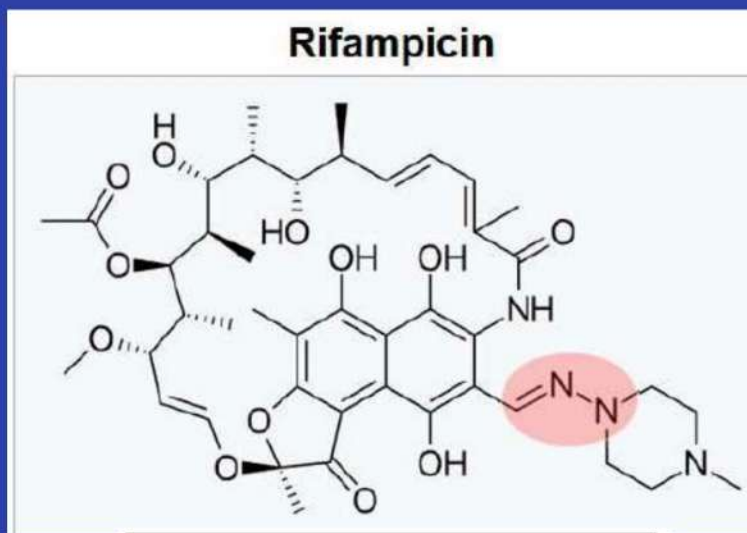
Scheme 1. Proposed Formation Mechanism of the E- and Z-Oximes of Irbesartan in the Reaction of Irbesartan with Nitrous Acid



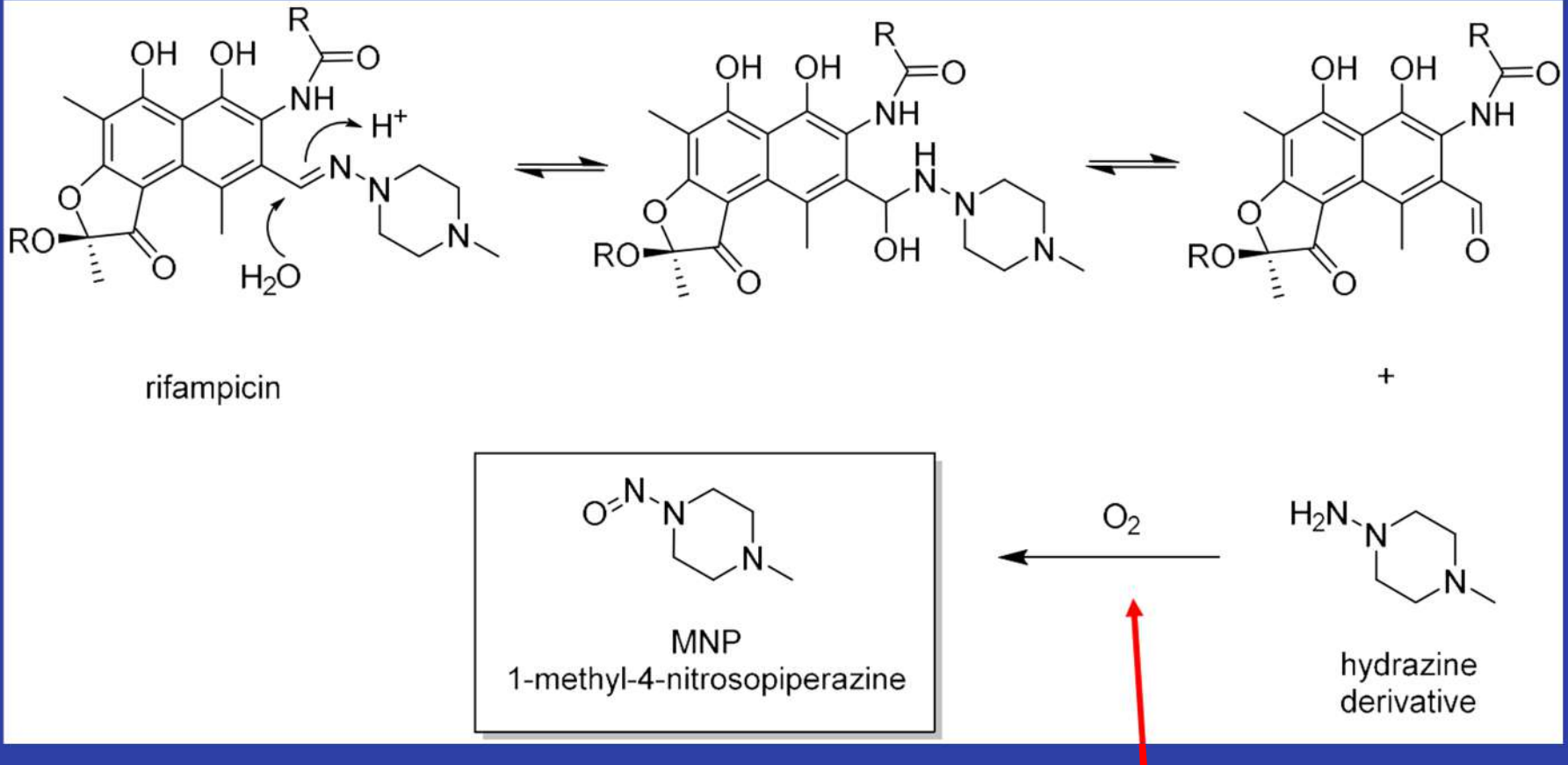
- Reaction of irbesartan with nitrous acid produces irbesartan oxime derivatives, rather than N-nitrosoirbesartan, OPRD, 26, 1236-1246 (2022)

N-Nitrosamine Impurities identified in Rifampicin and Rifapentine

- Aug 26, 2020. FDA became aware of these nitrosamine impurities



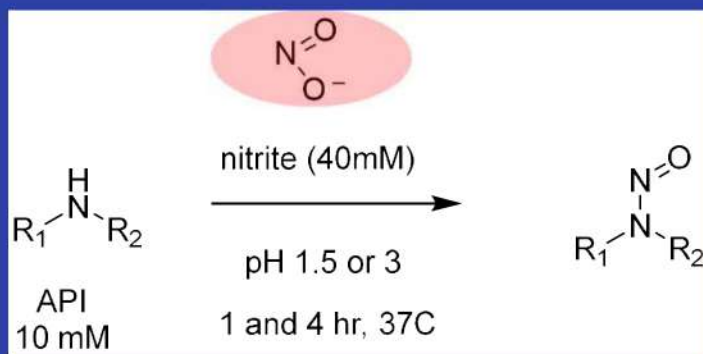
Possible Pathway?? – Implications...



Lunn G, Sansone EB, Andrews AW, Aerial Oxidation of Hydrazines to Nitrosamines, *Env. Mol. Mutagen.*, 17, 59-62 (1991)

N-Nitrosamines Risk Assessment – Can one be formed from “your” API: Two Approaches

➤ NAP Test¹



Problems with drug solubility
and artefact formation

➤ Approach 1: “Modified NAP Test”²



Proposed to be more effective

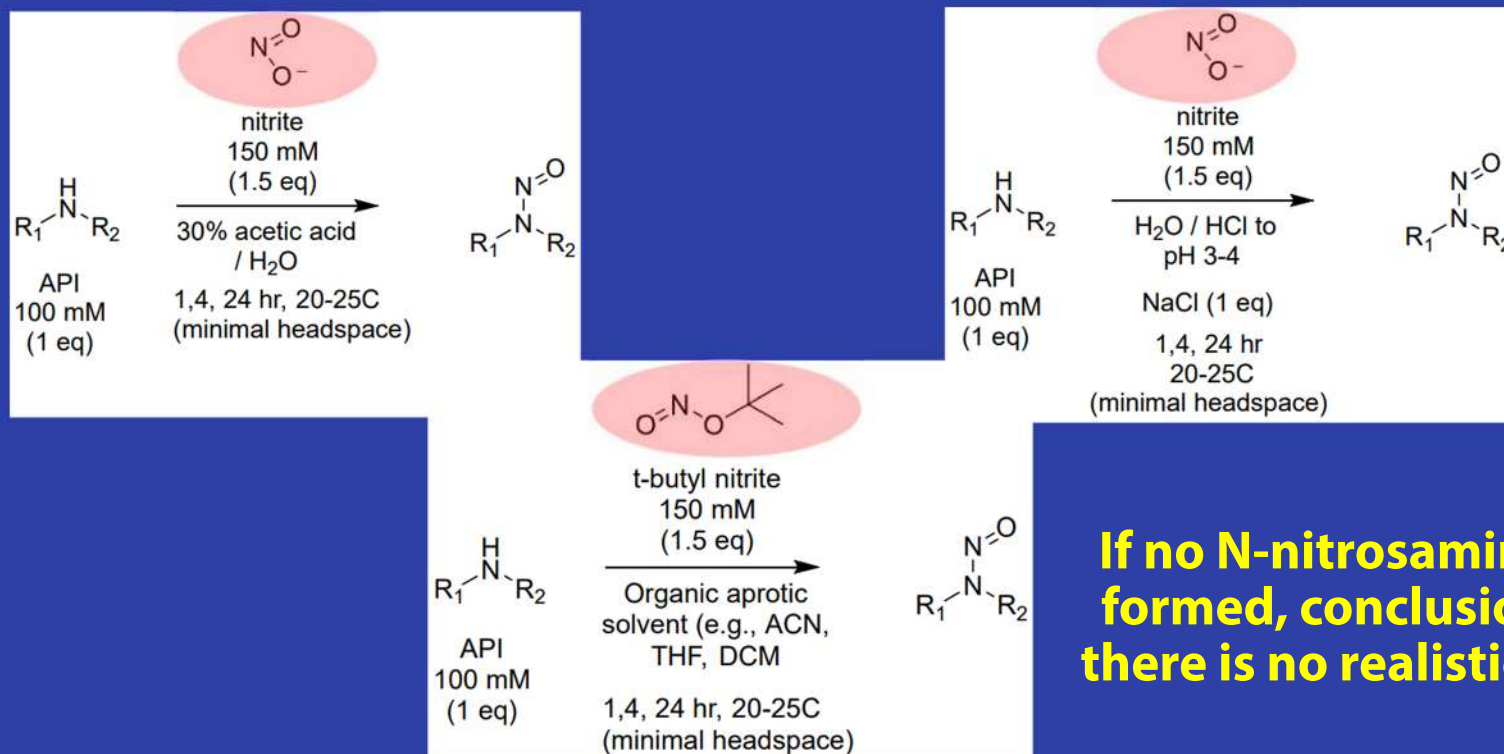
**If no N-nitrosamine is formed,
conclusion is there is no realistic risk**

¹Coulston F (1980), in “The potential carcinogenicity of nitrosatable drugs”, Coulston F, Dunne JF, Eds, Proceedings of a WHO Symposium, Geneva, June (1978), p. 8, Albex Publishing Co, Norwood, NJ USA

²Sharma N et al., Modified NAP test: A simple and responsive nitrosating methodology for risk evaluation of NDSRIs, J. Pharm. Sci., 112, 1333-1340 (2023)

N-Nitrosamines Risk Assessment – Can one be formed from “your” API: Two Approaches

- Approach 2: Application of 3 different “synthetic” conditions*



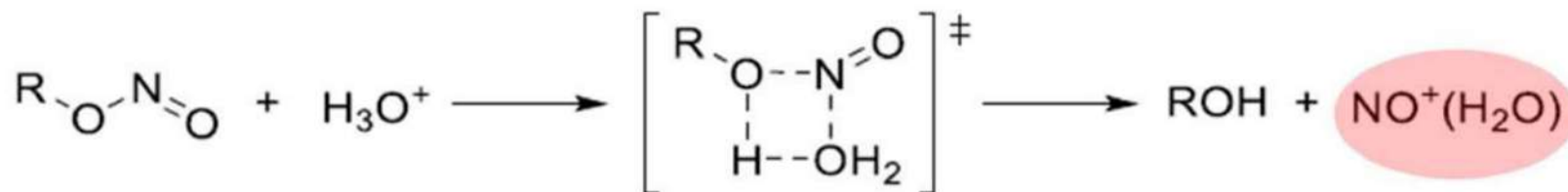
If no N-nitrosamine is formed, conclusion is there is no realistic risk

*Approaches and considerations for the investigation and synthesis of N-nitrosamine DS-related impurities (NDSRIs), Ashworth IA, Org. Proc. Res. Dev., 27, 1784-1791 (2023)

Alkyl nitrites: Nitrosating Agents

- Alkyl nitrites readily undergo acid-catalyzed hydrolysis in aqueous acidic conditions, generating nitrous acid-like nitrosating agents (Scheme 23).
- For TBN (t-butyl nitrite), the hydrolysis is so fast that the kinetics are identical with those when in situ formed nitrous acid is used.
- No difference using alkyl nitrites under these conditions over the use of $\text{NaNO}_2/\text{acid}(\text{aq})$

Scheme 23. Alkyl Nitrite Hydrolysis in Acid Aqueous Media



- *Pathways for N-nitroso compound formation: secondary amines and beyond, Lopez-Rodriguez R et al., Org. Proc. Res. Dev., 24, 1558-1585 (2020)

Formation of N-Nitrosamines in Drug Products: Assessing the Risk

- For solid dosage forms, physical contact needed for reaction to occur
 - Quantitative reaction of all nitrite present to form nitrosamines seems unlikely
 - Model SS experiments in formulated products performed by Pfizer and Merck researchers show plateauing of N-nitrosation @~10% of spiked

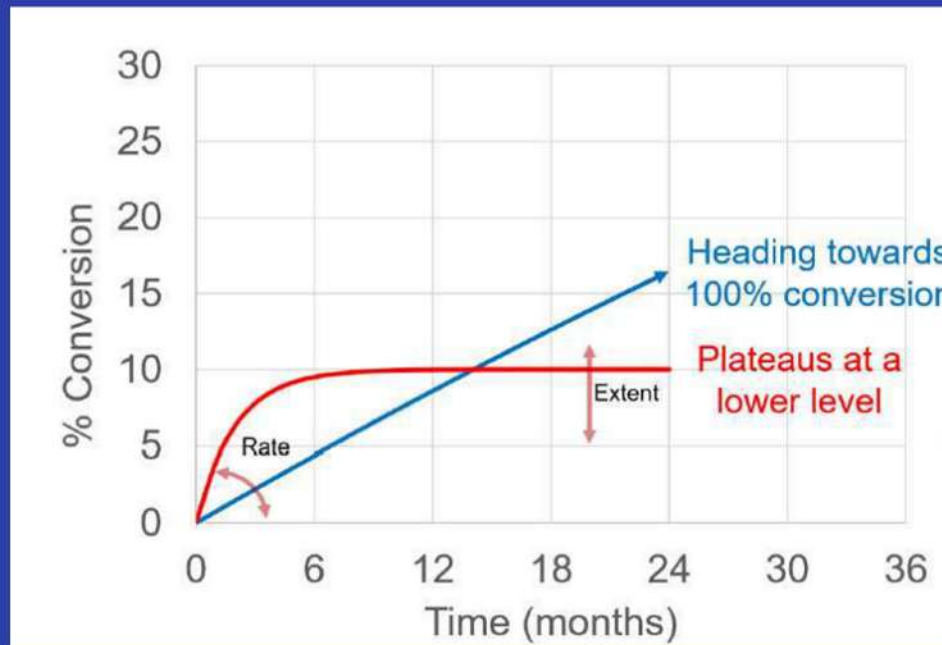


Figure adapted from G. Scrivens (Pfizer), presented at Science of Stability 2021

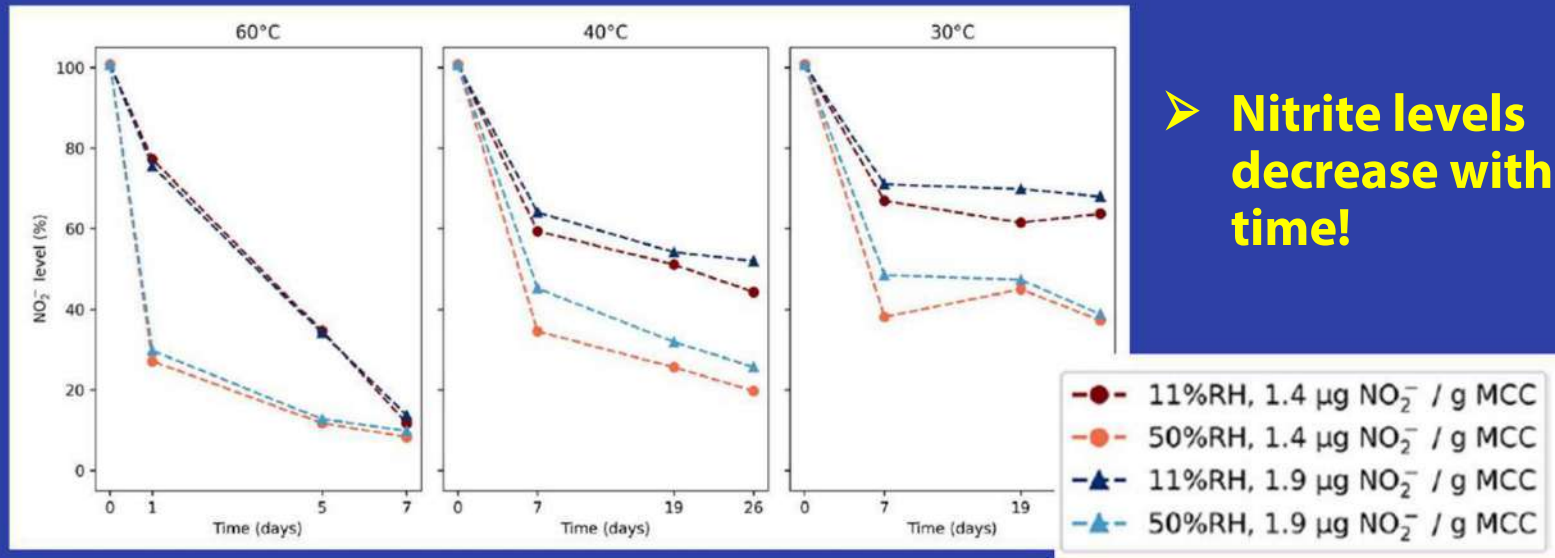
Commonly seen in Solutions

Commonly seen in solids

Model experiments appear to have plateauing behaviour

Formation of N-Nitrosamines in Drug Products: Assessing the Risk

- The salt form of nitrite can affect the N-nitrosation rate and plateau level
- **NH_4NO_2 , not NaNO_2 may be the predominant form** of “native” nitrite in excipients
- Empirical models **1.6% of “native” nitrite converted into N-nitrosamines**
- At high chloride concentrations, NOCl may be a relevant N-nitrosating reagent



Solid State Kinetics of Nitrosation Using Native Sources of Nitrite, Carloni L-E et al., J. Pharm. Sci., Volume 112, Issue 5, May 2023, Pages 1324-1332

Modeling the Impact of Excipients: Assessing the Risk

- Open-access Excel-based calculator provided
- % Conversion of nitrite levels (using “realistic percentages” based on formulation / manufacturing variables) from literature

dosage (Supplementary Materials File—toolkit). The input data that must be inserted for the analysis are:

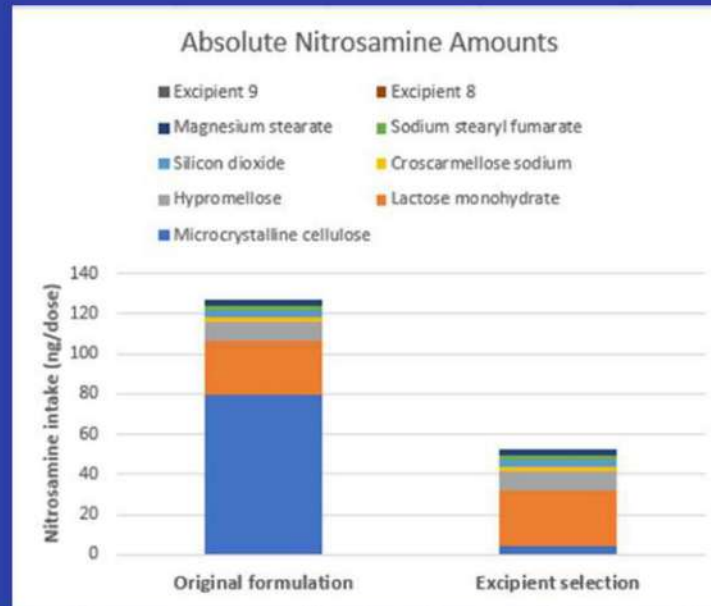
- The tablet dose weight;
- The dosage form composition (in percentage);
- The level of nitrite in each ingredient;
- The MW of the nitrosamine that is expected to form;
- The expected percentage of conversion of nitrite into nitrosamines.

Component	Concentration (% w/w)	Nitrite level (ug/g)	Nitrosamine amount (ng)	Relative amount (%)
API	15	0	0.00	0.00%
Microcrystalline cellulose	50	0.7	79.12	62.30%
Lactose monohydrate	22.5	0.54	27.47	21.63%
Hypromellose	5	0.8	9.04	7.12%
Croscarmellose sodium	3	0.42	2.85	2.24%
Silicon dioxide	1	1.3	2.94	2.31%
Sodium stearyl fumarate	3	0.39	2.64	2.08%
Magnesium stearate	0.5	2.6	2.94	2.31%
Excipient 8			0.00	0.00%
Excipient 9			0.00	0.00%
Total	100		127.00	100.00%

Modeling the impact of excipients selection on nitrosamine formation towards risk mitigation, *Pharmaceutics* 2023, 15, 2015, Berardi A et al.

Modeling the Impact of Excipients: Assessing the Risk

- Open-access Excel-based calculator provided

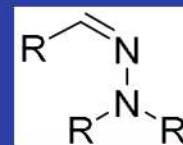


Amine type	Secondary amine			
	Direct compression (DC)		Wet granulation (WG)	
Manufacturing				
API form	Base	Salt	Base	Salt
Rate of conversion	10%	13%	9%	29%
Based on Moser et al. (2023)				

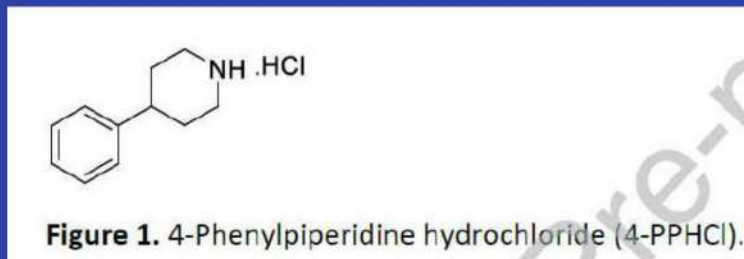
Modeling the impact of excipients selection on nitrosamine formation towards risk mitigation, *Pharmaceutics* 2023, 15, 2015, Berardi A et al.

Formation of N-Nitrosamines in Drug Products: Assessing the Risk

- Focus on APIs containing **secondary amines as a higher risk**, then on **tertiary amines / quaternary salts as a lower risk**
- Do not overlook scaffolds analogs that could hydrolyze to hydrazines
- Low level deg products should be considered, but it is difficult to envision a significant risk
 - Regulatory agencies still seem to consider this a risk
- **The pH of the formulation** (microenvironmental pH for solid formulations) is a critical aspect (2.5-3.4 is optimum for reaction)



Inhibition of N-Nitrosamine Formation in Drug Products

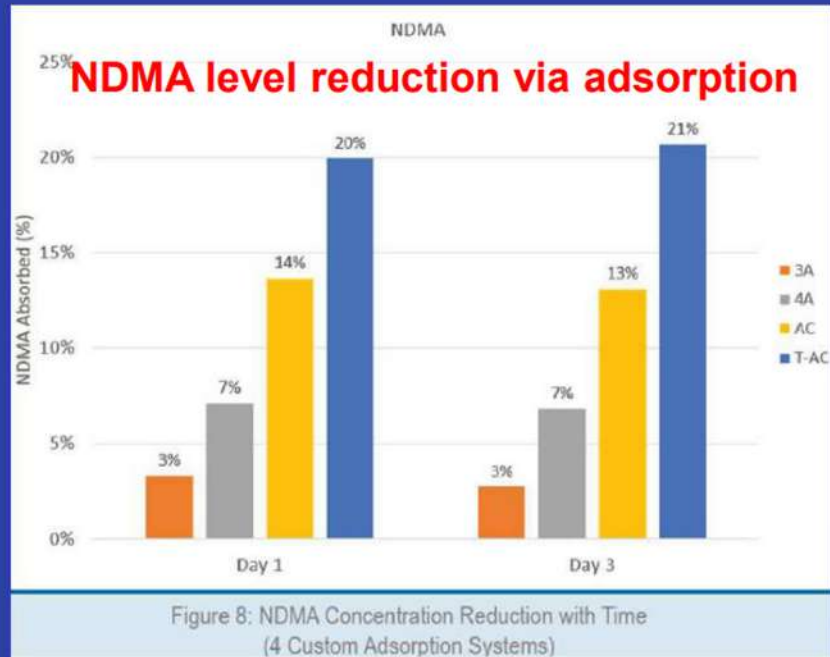
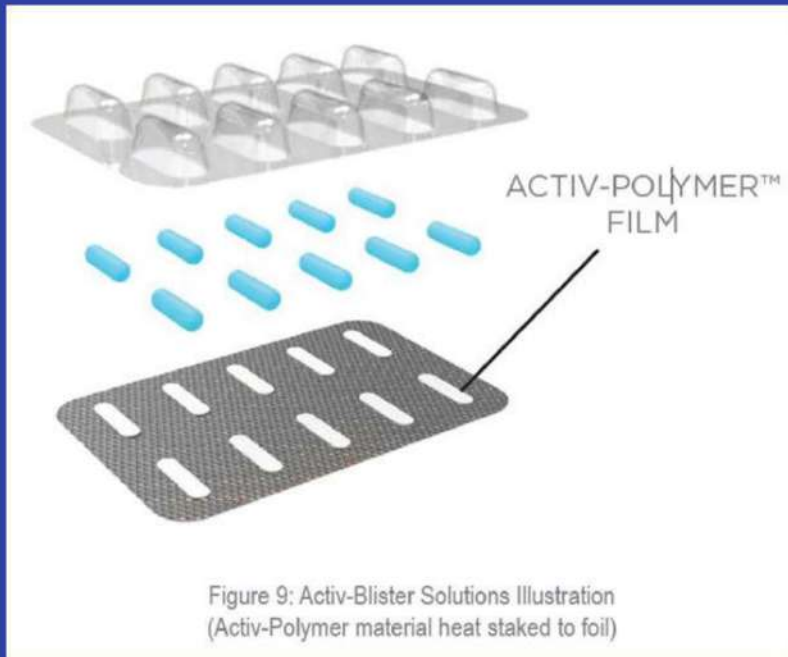


- **Inhibition of N-Nitrosamine Formation in Drug Products: A Model Study**, Nanda KK et al., J. Pharm. Sci., 110:12, 3773-3775 (2021)
 - Model compound (4-phenylpiperidine HCl) used, spiked into 100 mg tablets (wet granulation) at a 10% w/w load.
 - ✓ Storage at 50C / 75% RH, 1 month
 - ✓ Five inhibitors investigated – **ascorbic acid, sodium ascorbate, alfatocopherol, caffeic acid, and ferulic acid. All showed >80% inhibition when spike at ~1% w/w level.**
 - ✓ Potential use of amino acids – **glycine, lysine, and histidine** demonstrated in solution
- **Stay tuned! Garry Scriven's Nov 14 webinar** will expand on this topic!!

Inhibition of N-Nitrosamine Formation in Drug Products

➤ Packaging Options?

- Desiccants, Oxygen Scavengers, Custom Adsorption Systems?

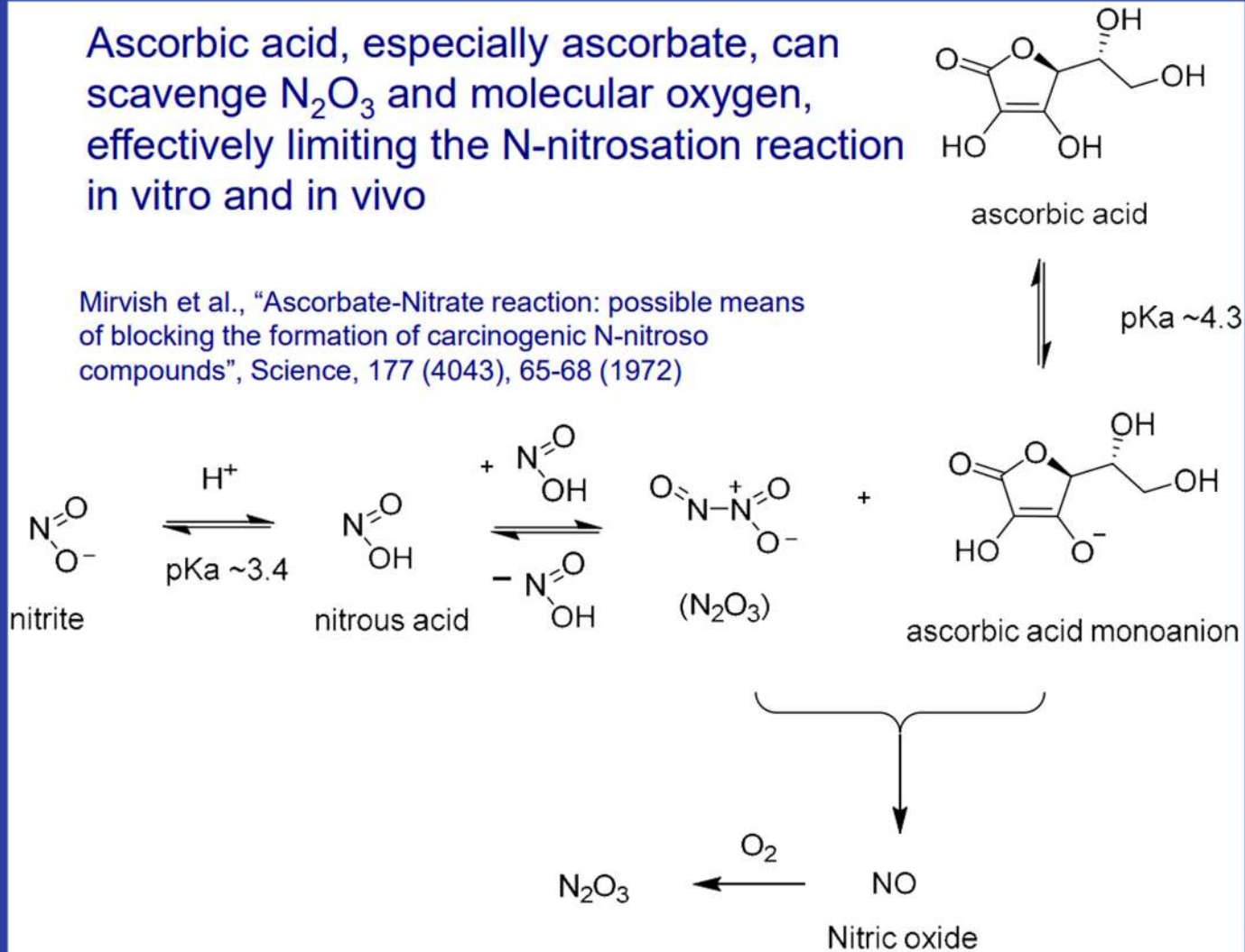


Mitigating N-nitrosamine risks with novel active material science innovations, technical white paper, csptechnologies.com

Inhibiting N-Nitrosation Using Ascorbic Acid

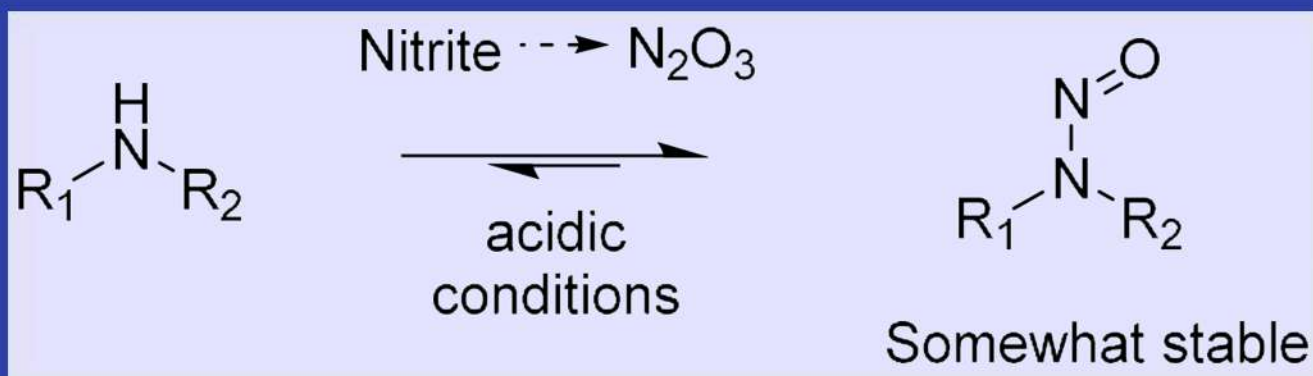
Ascorbic acid, especially ascorbate, can scavenge N_2O_3 and molecular oxygen, effectively limiting the N-nitrosation reaction in vitro and in vivo

Mirvish et al., "Ascorbate-Nitrate reaction: possible means of blocking the formation of carcinogenic N-nitroso compounds", *Science*, 177 (4043), 65-68 (1972)



Stability / Degradation of N-Nitrosamines

- The chemical stability of N-nitrosamines can be highly variable¹
 - Some compounds **reverting to parent or degrading within hours**
 - Other compounds can have **"enduring stability"**



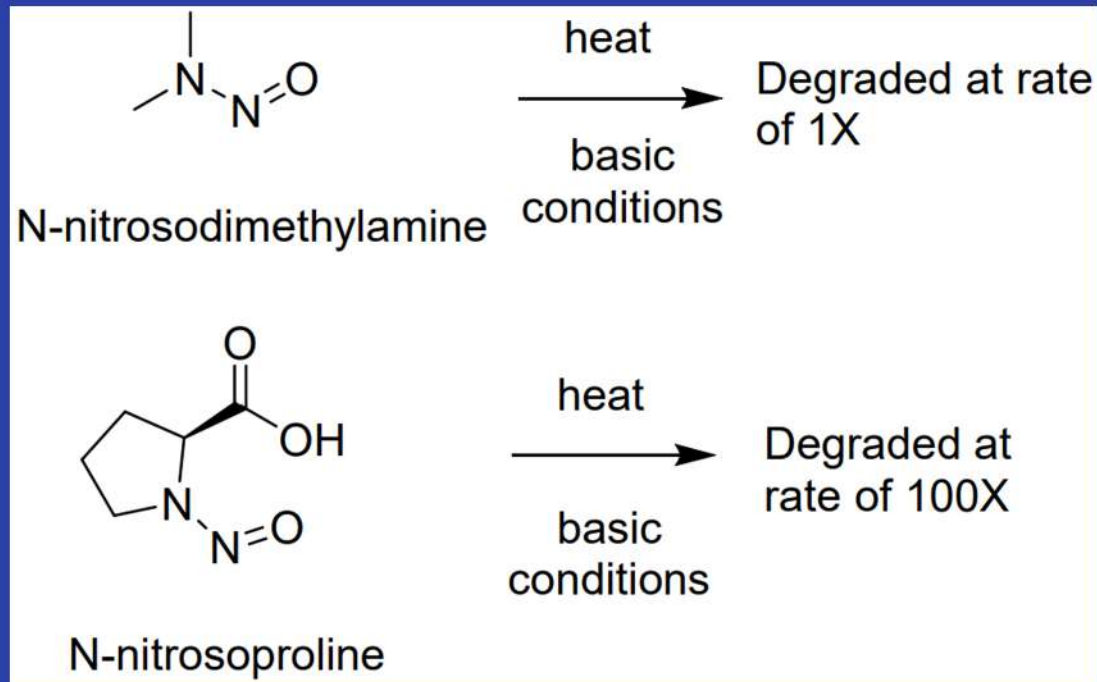
- The N-NO bond is, in general, readily dissociated at high temperatures²
 - Exposure to high temps – 400-500C
 - Led to development of the **thermal energy analyzer** in the 1970s as a highly sensitive detector for N-Nitrosamines

¹Ashworth IA, Org. Proc. Res. Dev., 27, 1784-1791 (2023)

²Lessons learnt from presence of N-nitrosamine impurities in sartan medicines, 23 June 2020 EMA/526934/2019

Stability / Degradation of N-Nitrosamines

- The chemical stability of N-nitrosamines can be highly variable¹
 - At 110 °C and alkaline pH (8.5 to 12.5)
 - Cyclic Nitrosamines degraded **100X faster** than straight chain Nitrosamines²



¹Ashworth IA, Org. Proc. Res. Dev., 27, 1784-1791 (2023)

²Fan, T.Y.; Tannenbaum, S.R. 1972. Stability Of N-Nitroso Compounds. Journal Of Food Science, 37, 274-276.

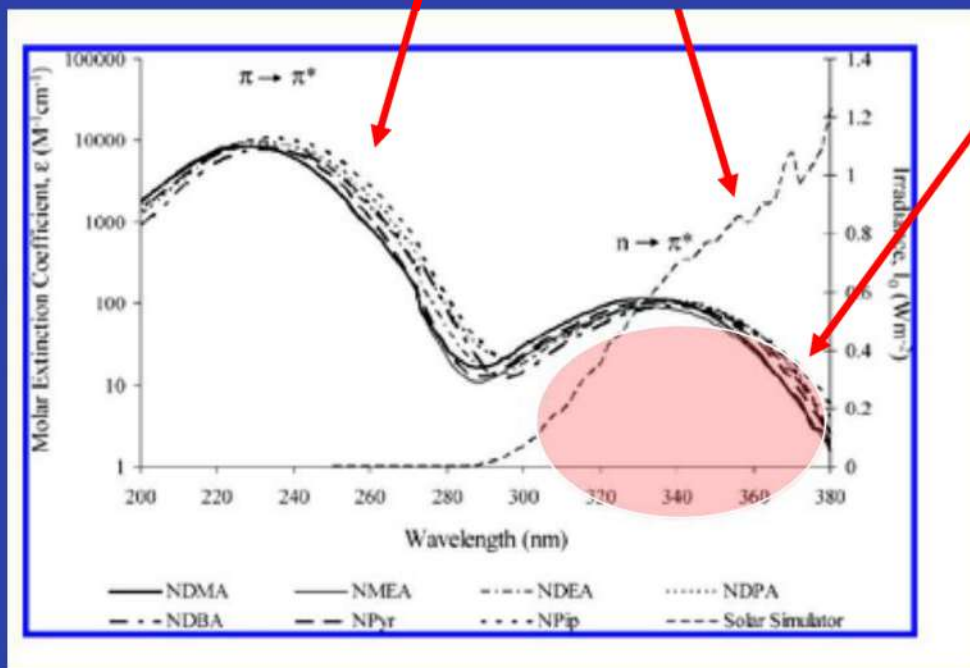
Stability / Degradation of N-Nitrosamines

➤ N-nitrosamines in general are **photo-labile**

Absorbance of N-nitrosamines

Solar Emission

Overlap integral in the UVA region



- N-nitrosamine photolysis in sunlight in water is fast
- **Half-lives of ~ 15 min!!**
- Quantum yields ranging from 0.4-0.6 (Table 8; Plumlee and Reinhard, 2007)

¹Plumlee, M.H.; Reinhard, M. 2007. Photochemical attenuation of N-nitrosodimethylamine (NDMA) and other nitrosamines in surface water. Environ. Sci. Technol. 41, 6170-6176.

Conclusions

- ✓ **The potential formation of low levels of N-nitrosamines in formulated products should not be ignored**
- ✓ **Residual nitrites in excipients appear to be the main nitrosating agent in formulated products**
 - ✓ **Quantitative reaction of all nitrite present to form nitrosamines is unlikely**
- ✓ **Microenvironmental pH of the formulation is a critical factor**
- ✓ **Inhibitors in the formulation and/or packaging configurations may be viable approach**
- ✓ **Chemistry understanding can help focus and guide the risk assessment**

Thank you!!



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