Use of an *in silico* tool to determine the molecular susceptibility of compounds forming nitrosamine degradation products

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Lhasa Limited

We are a Not-For-Profit Organisation and Educational Charity

To enable informed decision making on chemical safety

Objective

We create cutting-edge software technology which streamlines compound development and minimises animal testing.

Members

(599 globally)

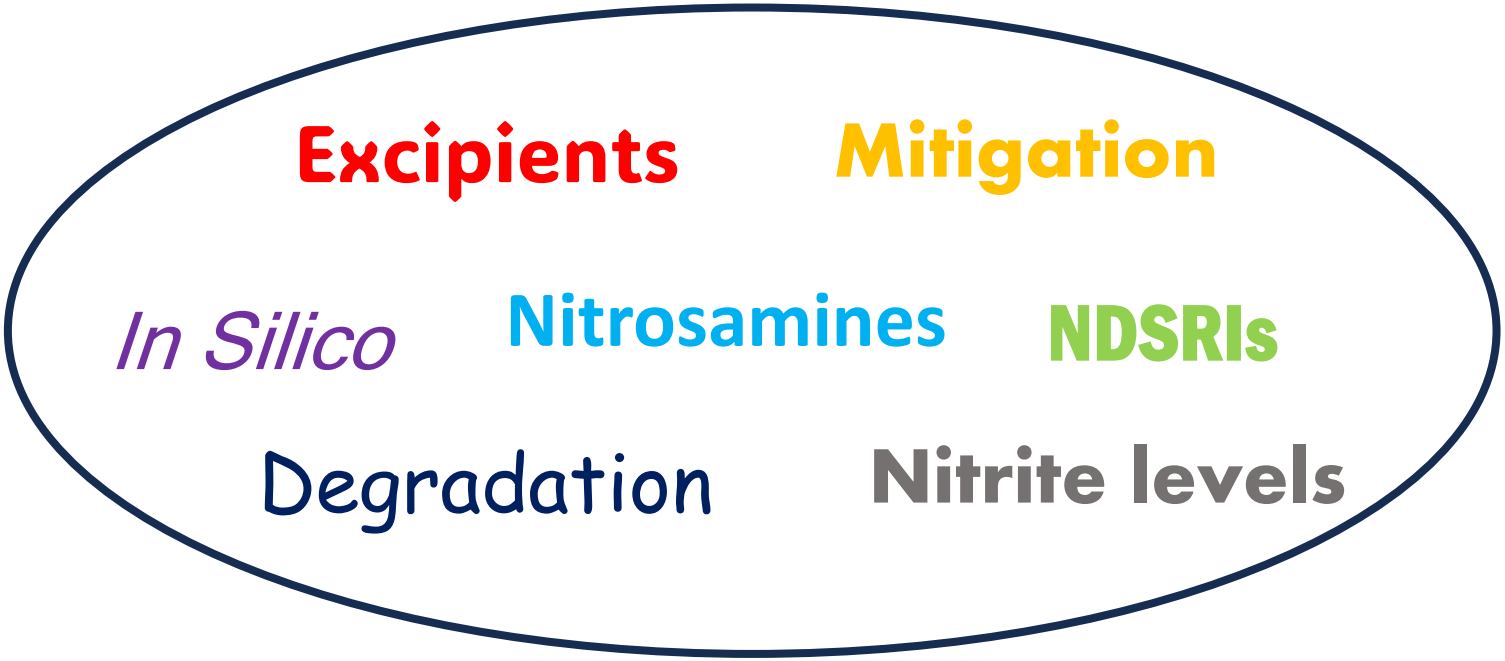
Our technology is developed in collaboration with industry stakeholders and regulators.

Software solutions

Derek nexus
Zeneth
Meteor nexus
Sarah nexus
Mirabilis
Vitic
Kaptis

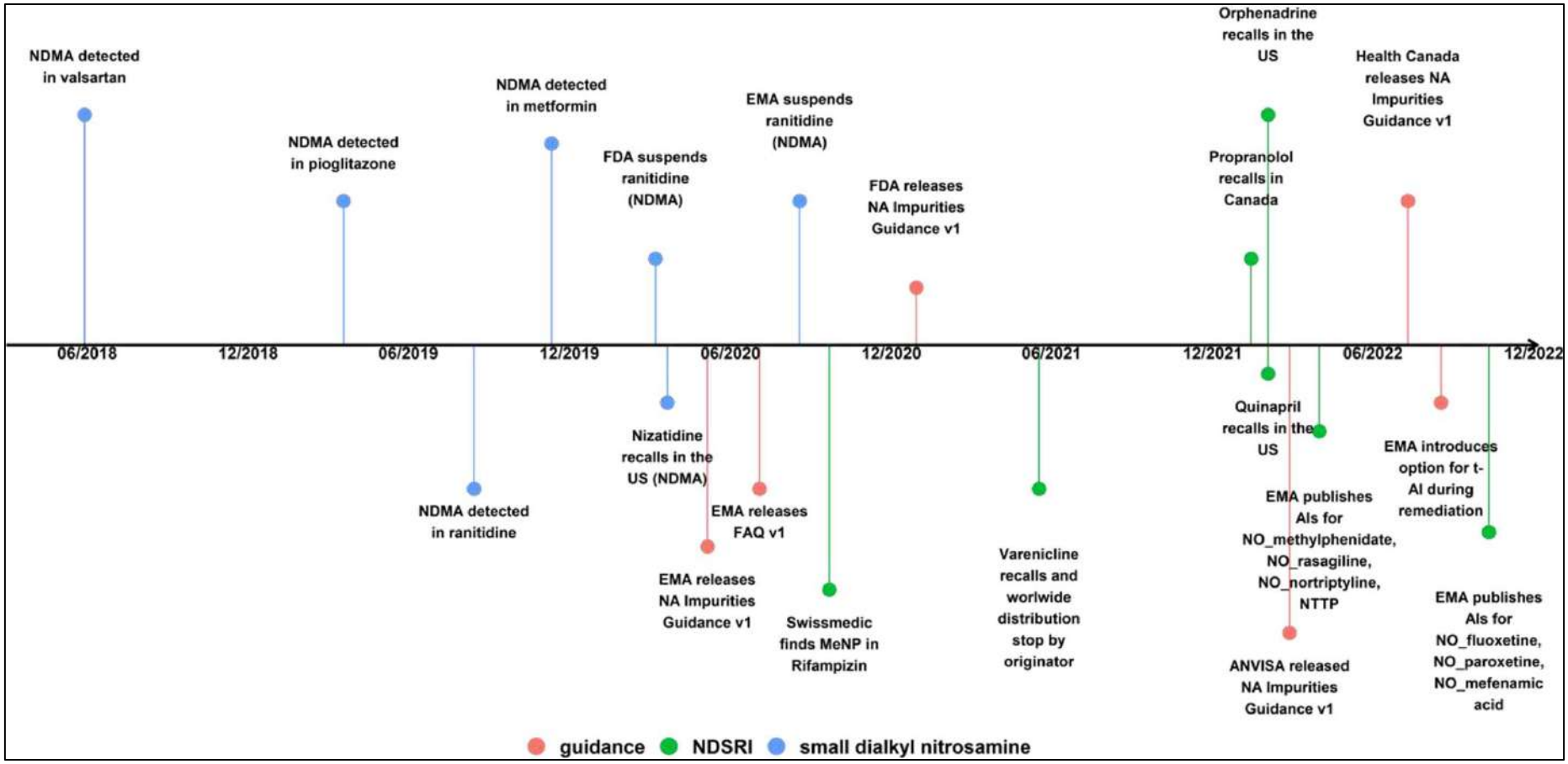
Lhasa Limited | Shared Knowledge, Shared Progress

Keywords



Lhasa Limited | Shared Knowledge, Shared Progress

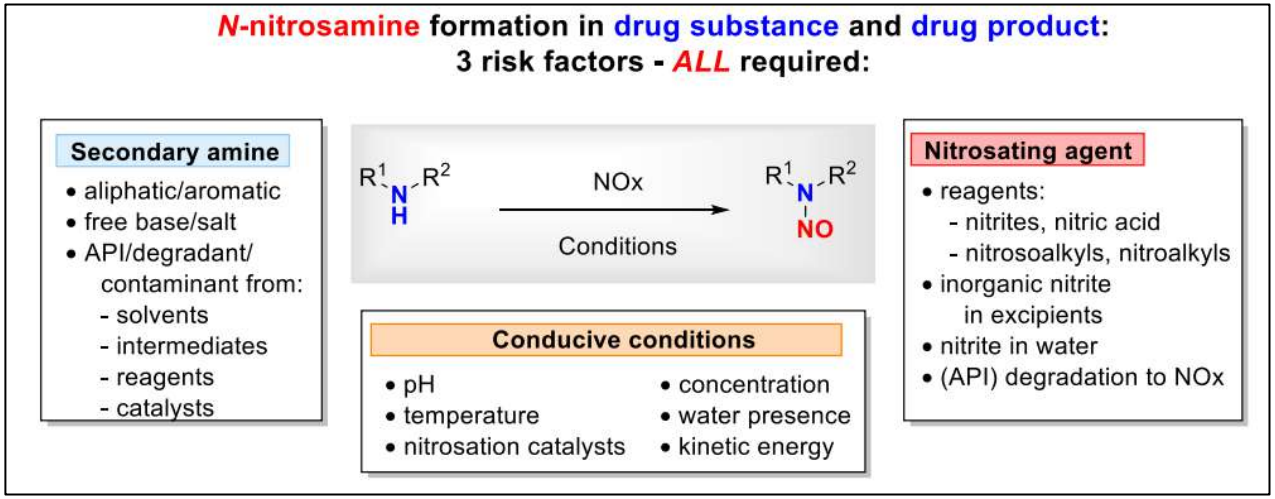
The Nitrosamine Saga[1]



1. The Nitrosamine Saga: Lessons learned from five years of scrutiny, R. Nudelman et al, Org. Process Res. Dev., 2023, in press.

Challenges: what, where, who?

What?



2. Formation of *N*-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies, Cioc et al, Org. Process. Res. Dev., 2023, in press.

Where?

- ✓ Route of synthesis API
- ✓ Drug manufacturing process
- ✓ Degradation – DS or DP (DS + Impurity)
- ✓ Primary packaging materials

Who?

Zeneth

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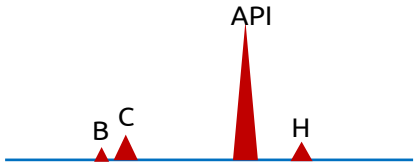
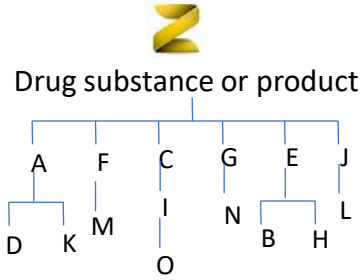
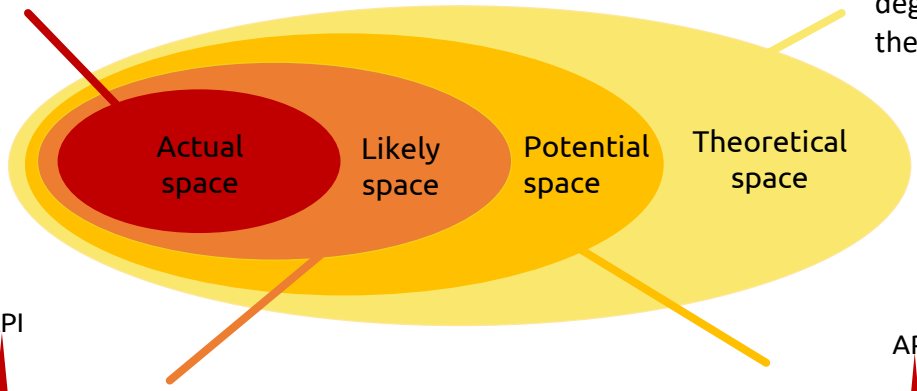
How can an *in silico* tool help?

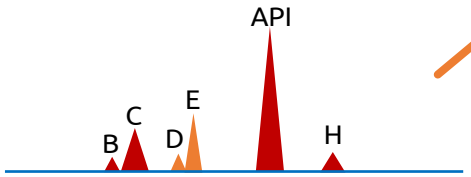


Actual degradation products as seen in final packaging and storage conditions

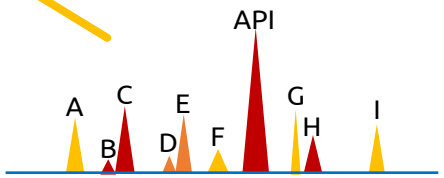


In silico theoretically predicted degradation products aim to inform the potential space



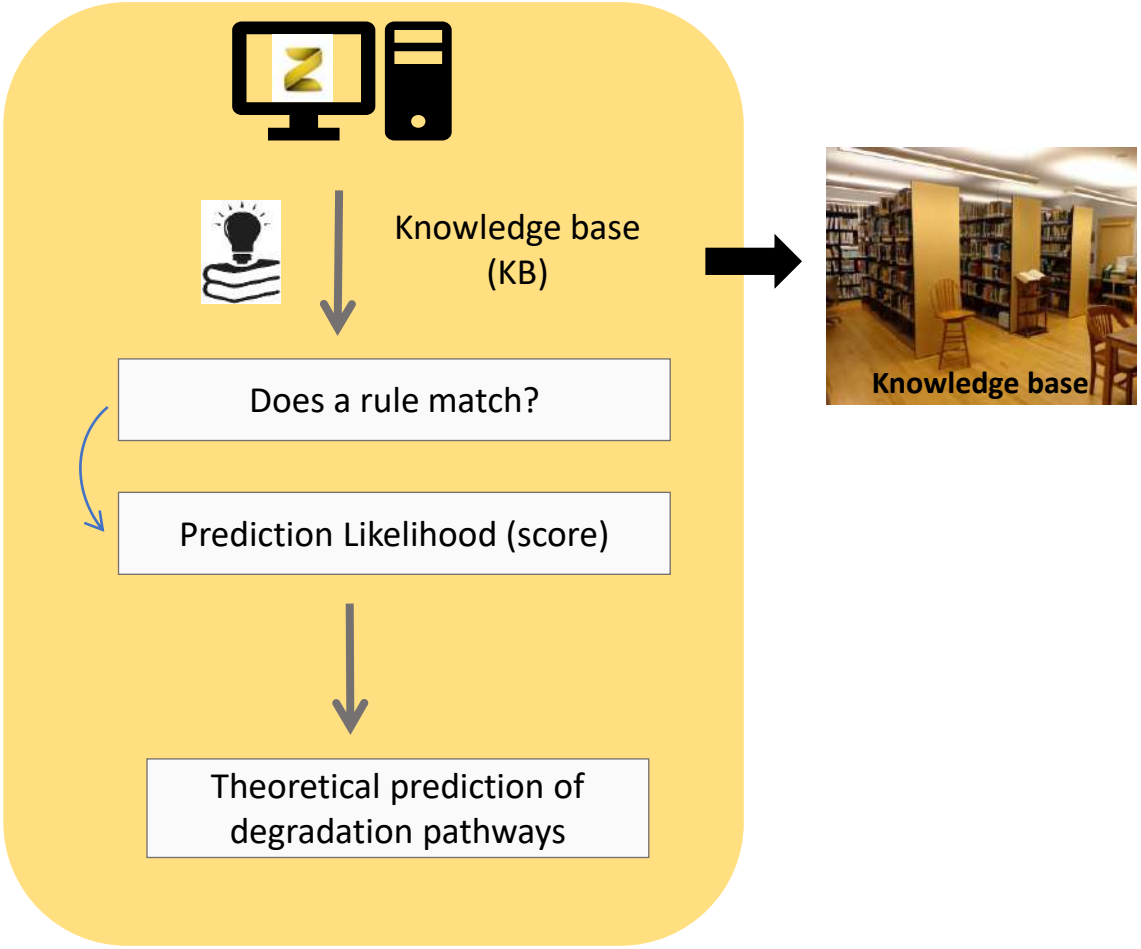


Accelerated stability studies aim to identify likely degradation products



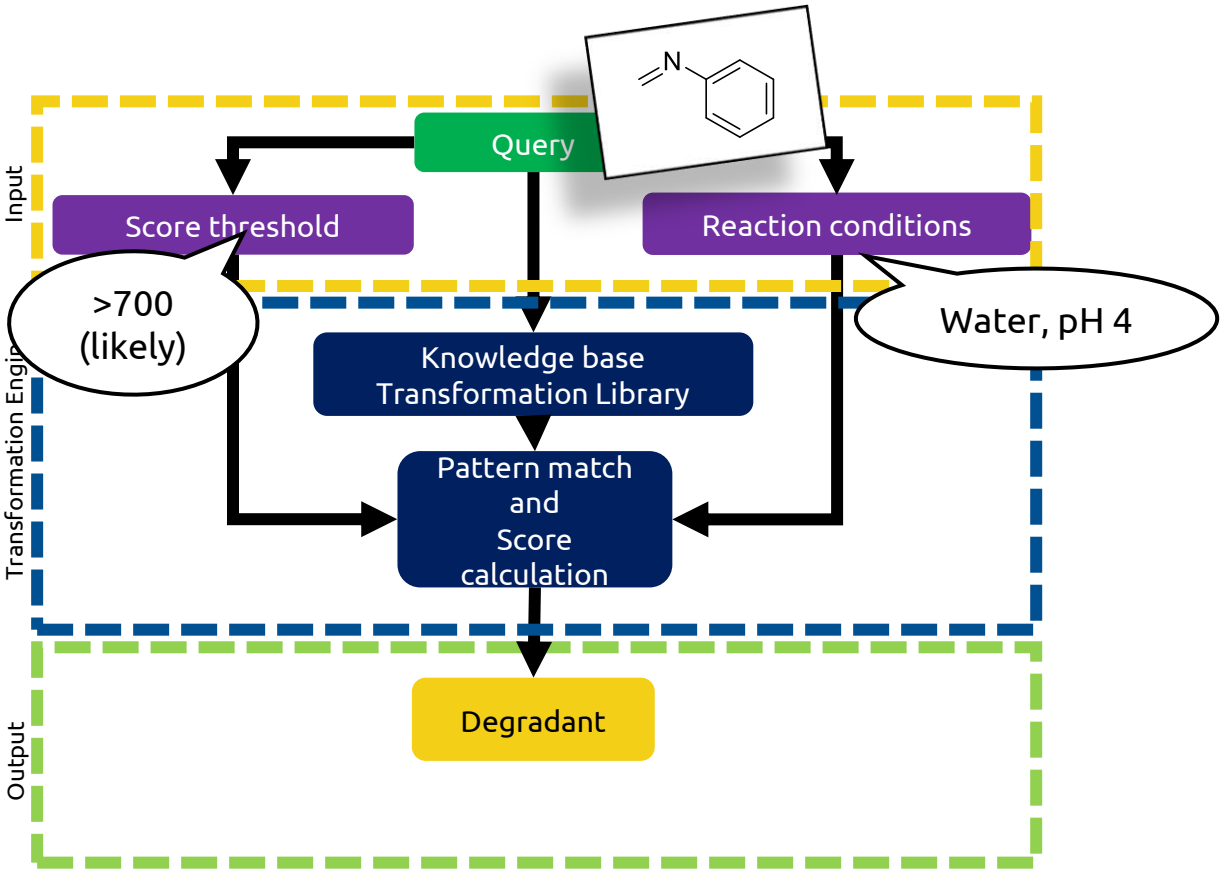
Forced degradation studies aim to identify potential degradation products

How does this tool work?[3,4]

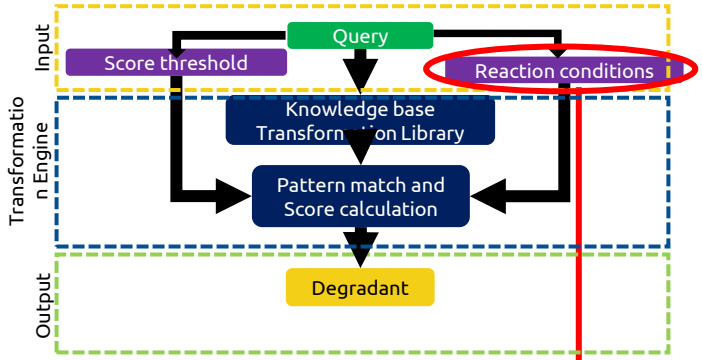


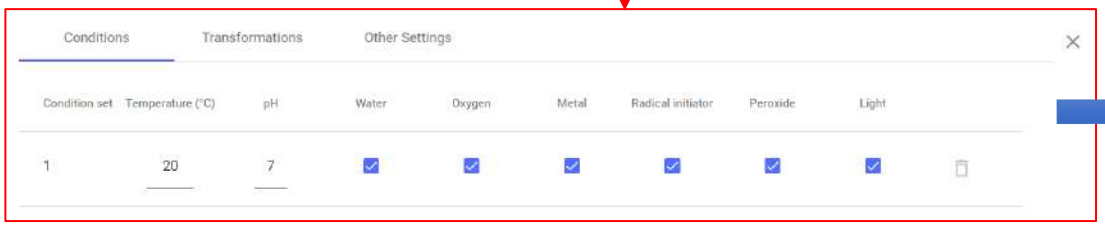
3. An expert system to predict the forced degradation of organic molecules, Parenty et al, Mol. Pharm., 2013, 10, 2962-2974.
4. Chapter 3: In silico drug degradation prediction. Ali et al, in: Methods for Stability Testing of Pharmaceuticals. Editors: Bajaj and Singh, 2018, pp 53-73.

Methodology



Methodology



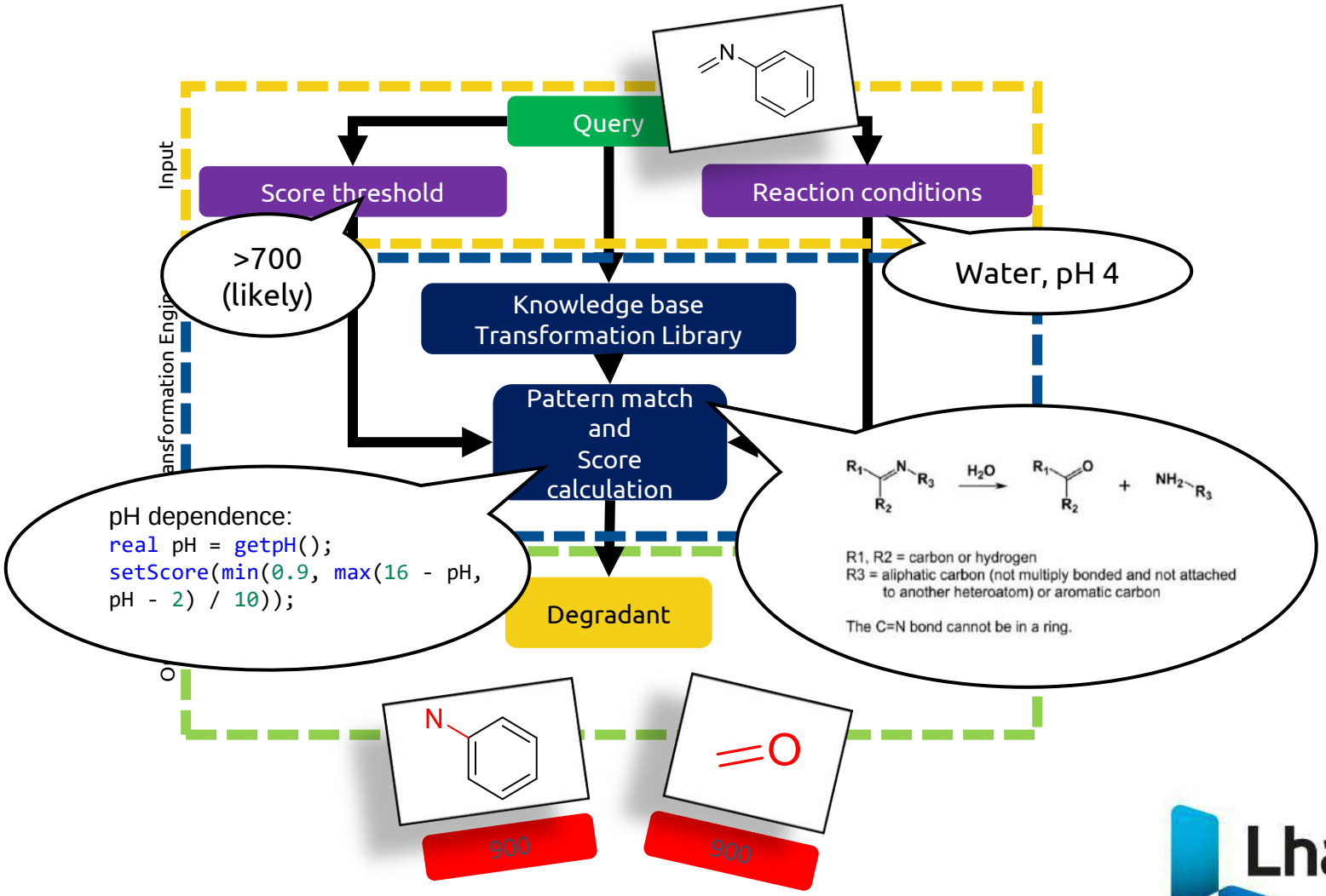


*Information from Q3B guideline

CONDITIONS GENERALLY EMPLOYED FOR FORCED DEGRADATION

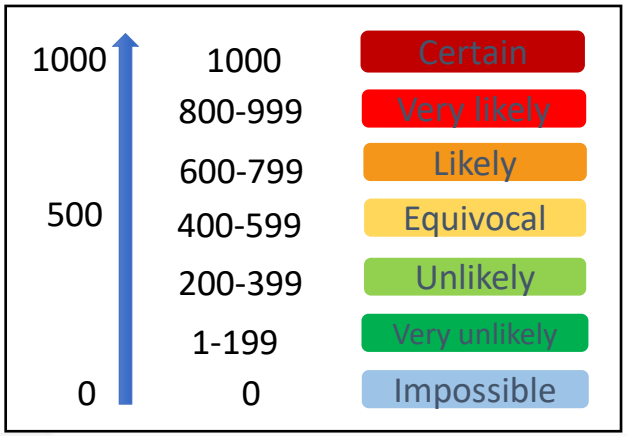
Degradation Type	Experimental Condition	Storage Condition	Sampling Time
Hydrolysis	Control API (no acid or base)	40 °C, 60 °C	1, 3, 5 days
	0.1N HCl	40 °C, 60 °C	1, 3, 5 days
	0.1N NaOH	40 °C, 60 °C	1, 3, 5 days
	Acid Control (no API)	40 °C, 60 °C	1, 3, 5 days
	Base Control (no API)	40 °C, 60 °C	1, 3, 5 days
	pH: 2, 4, 6, 8	40 °C, 60 °C	1, 3, 5 days
Oxidative	3% H_2O_2	25 °C, 40 °C	1, 3, 5 days
	Peroxide Control	25 °C, 40 °C	1, 3, 5 days
	Azobisisobutyronitrile (AIBN)	40 °C, 60 °C	1, 3, 5 days
	AIBN Control	40 °C, 60 °C	1, 3, 5 days
Photolytic	Light, 1 X ICH	NA	1, 3, 5 days
	Light, 3 X ICH	NA	1, 3, 5 days
	Light control	NA	1, 3, 5 days
Thermal	Heat Chamber	60 °C	1, 3, 5 days
	Heat Chamber	60 °C / 75% RH	1, 3, 5 days
	Heat Chamber	80 °C	1, 3, 5 days
	Heat Chamber	80 °C / 75% RH	1, 3, 5 days
	Heat Control	Room Temp.	1, 3, 5 days

Methodology



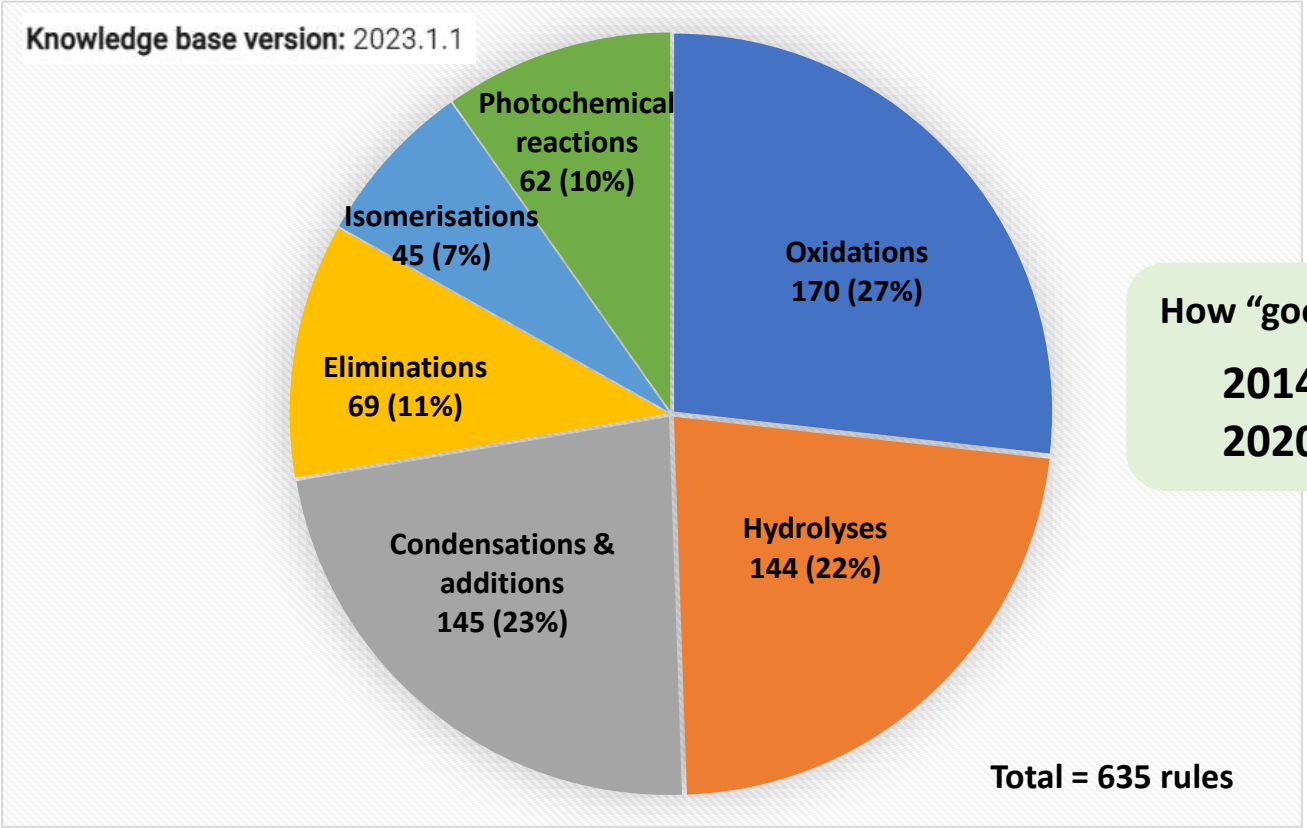
Output: Results tree

Representative example: API bromazepam



Knowledge base version: 2023.1.1

Coverage within Zeneth



How "good" is this knowledge base?

2014: 54% sensitivity [5]

2020: 70% sensitivity [6]

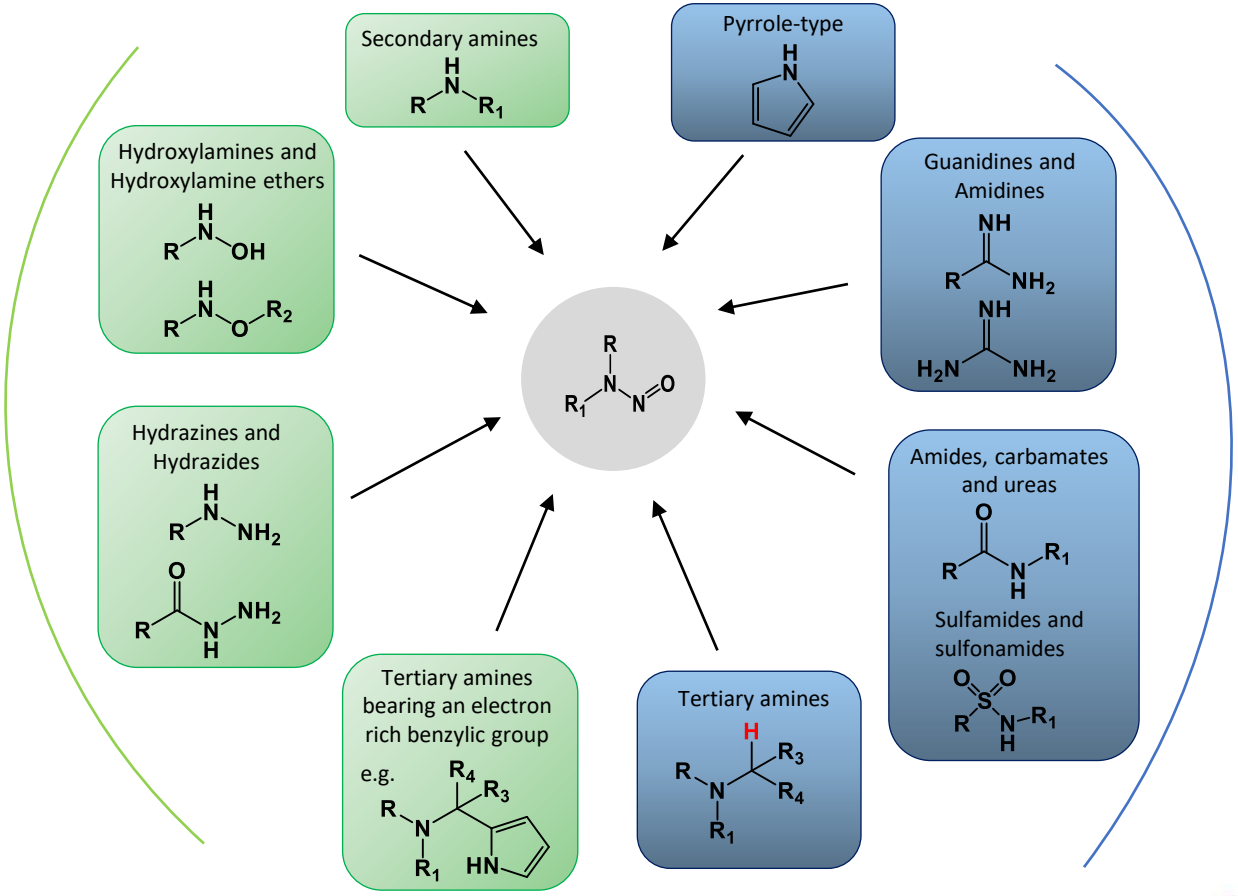
5. In Silico Prediction of Pharmaceutical Degradation Pathways: A Benchmarking Study, Kleinman et al, Mol. Pharm., 2014, 11, 4179-4188.

6. In silico prediction of pharmaceutical degradation pathways: a benchmarking study using the software program Zeneth, Hemingway et al, submitted for publication. ed.

Coverage of nitrosation reactions

N-N bond formation

- **Stronger nucleophiles**
- **Higher likelihood scores**

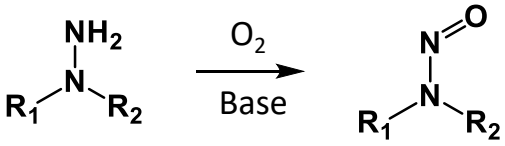


- **Weaker nucleophiles**
- **Lower likelihood scores**

Coverage of nitrosation reactions

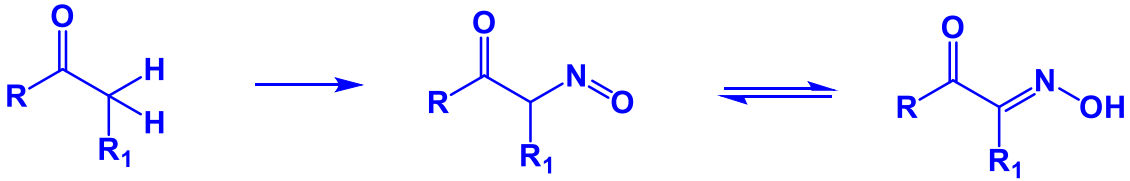
N-O bond formation

Hydrazines

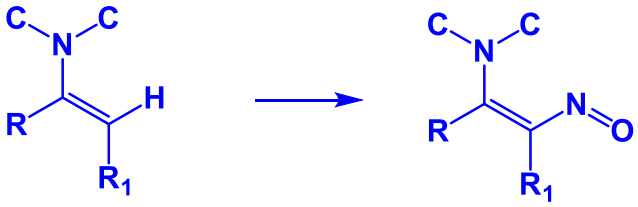


N-C bond formation

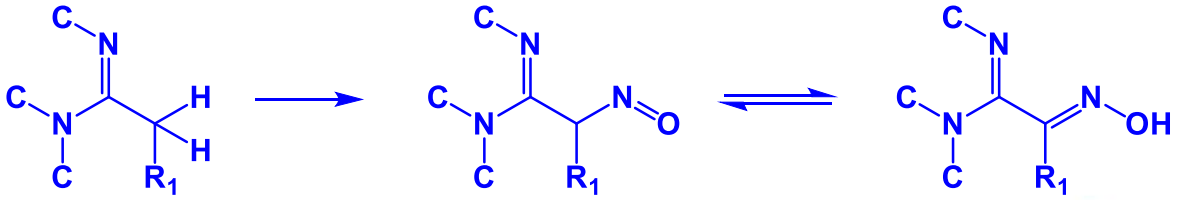
Enols



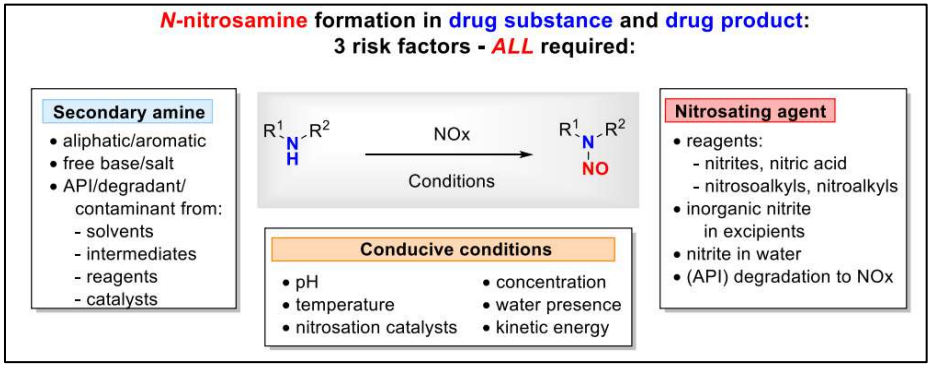
Enamines



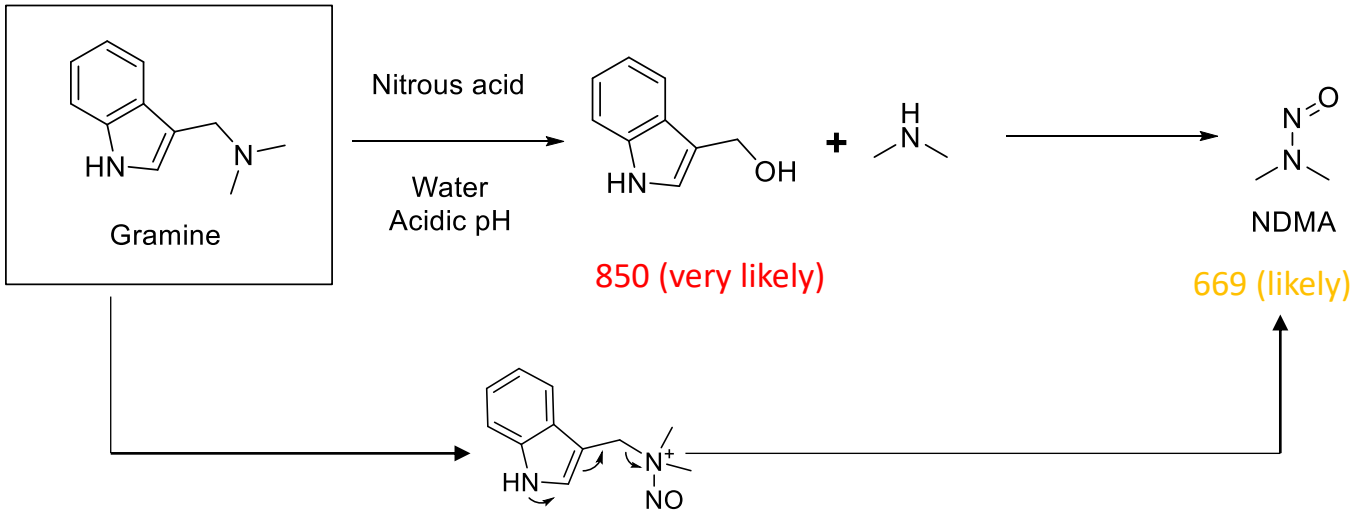
Amidines



Nitrosamine degradant generation



Zeneth



Zeneth → assess the theoretical potential of your API to form a nitrosamine

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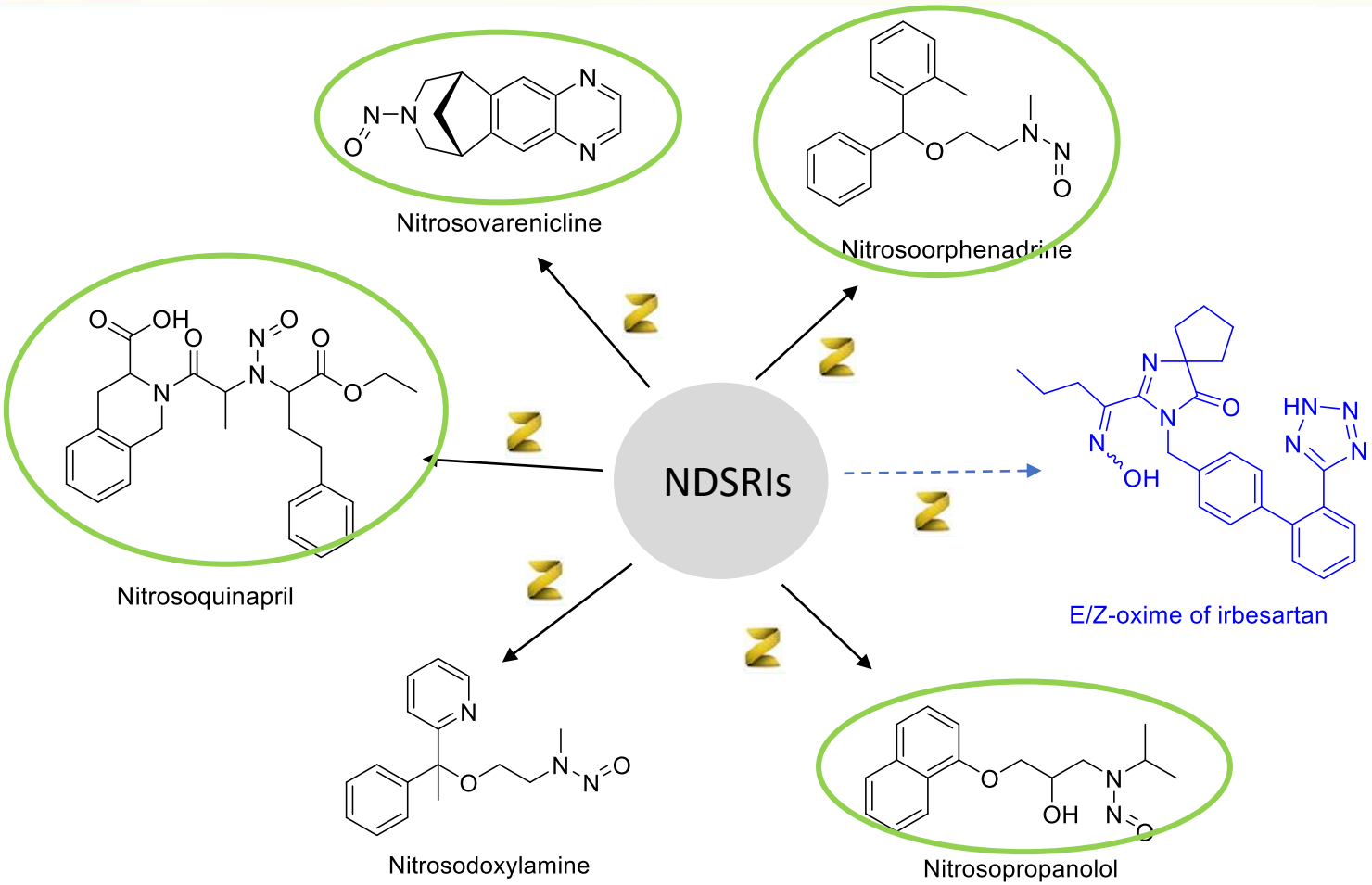
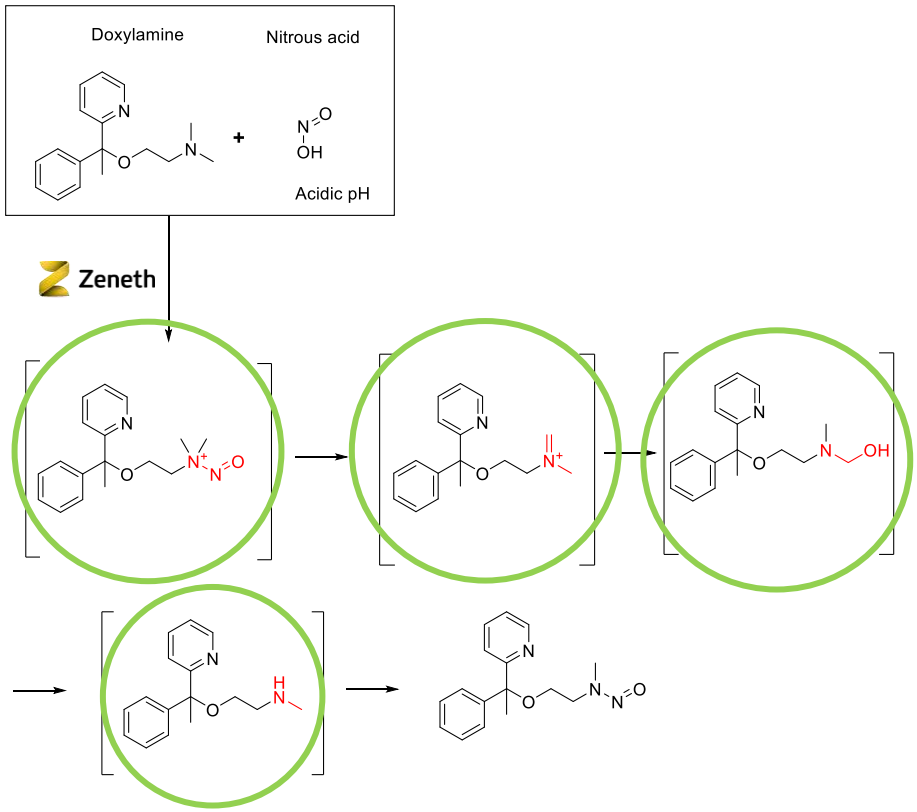
NDSRIs



Zeneth → assess the theoretical potential of your API to form an NDSRI

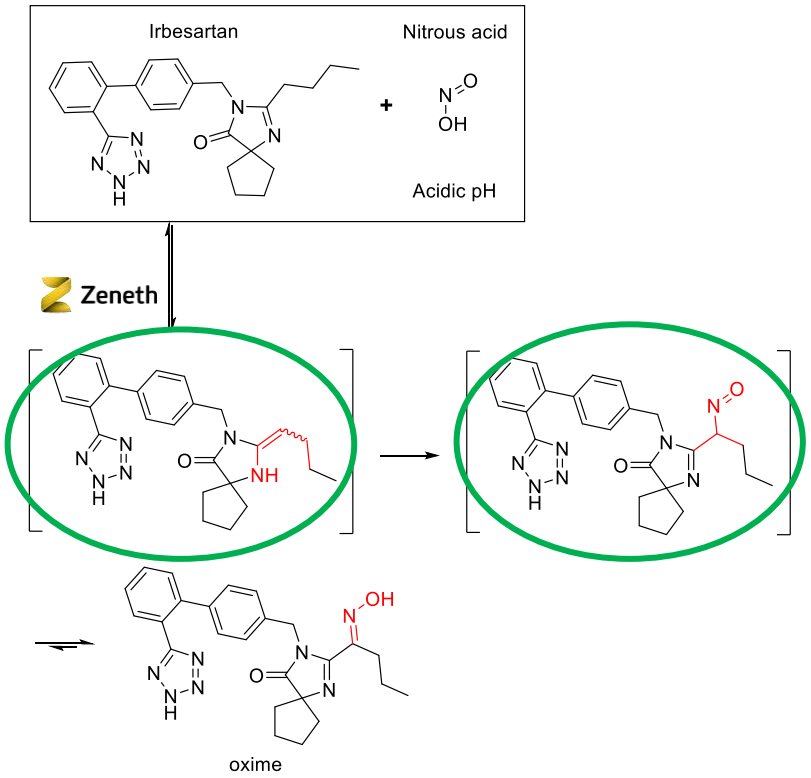
Predictions at acidic pH

Doxylamine: N-Nitrosation of a tertiary amine



7. Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond, Lopez-Rodriguez et al, Org. Process Res. Dev., 2020, 24, 1558-1585.

Irbesartan: C-Nitrosation of an amidine



8. Reaction of Irbesartan with Nitrous Acid Produces Irbesartan Oxime Derivatives, rather than *N*-Nitrosoirbesartan, Lin et al, Org. Process Res. Dev. 2022, 26, 4, 1236-1246.

The landscape

"In total, 40.4 % of the analyzed APIs and 29.6 % of the API impurities are potential nitrosamine precursors"

ELSEVIER

Journal of Pharmaceutical Sciences
Volume 112, Issue 5, May 2023, Pages 1287-1304

Global Health

The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals

Joerg Schlingemann [a 1], Michael J. Burns [b 1], David J. Ponting [b], Carolina Martins Avila [b f], Naiffer E. Romero [c], Mrunal A. Jaywant [c], Graham F. Smith [d], Ian W. Ashworth [e], Stephanie Simon [a], Christoph Saal [a], Andrzej Wilk [c]

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Regulators

"Cooperation between some **drug product manufacturers**, **marketing authorization holders** and **excipient suppliers** has allowed for a better understanding of the nitrite content of various excipients."

ORGANIC PROCESS RESEARCH & DEVELOPMENT

OPR&D

pubs.acs.org/OPRD

Review

Formation of *N*-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies

Răzvan C. Cioc, Ciarán Joyce, Monika Mayr, and Robert N. Bream*

Cite This: https://doi.org/10.1021/acs.oprd.3c00153

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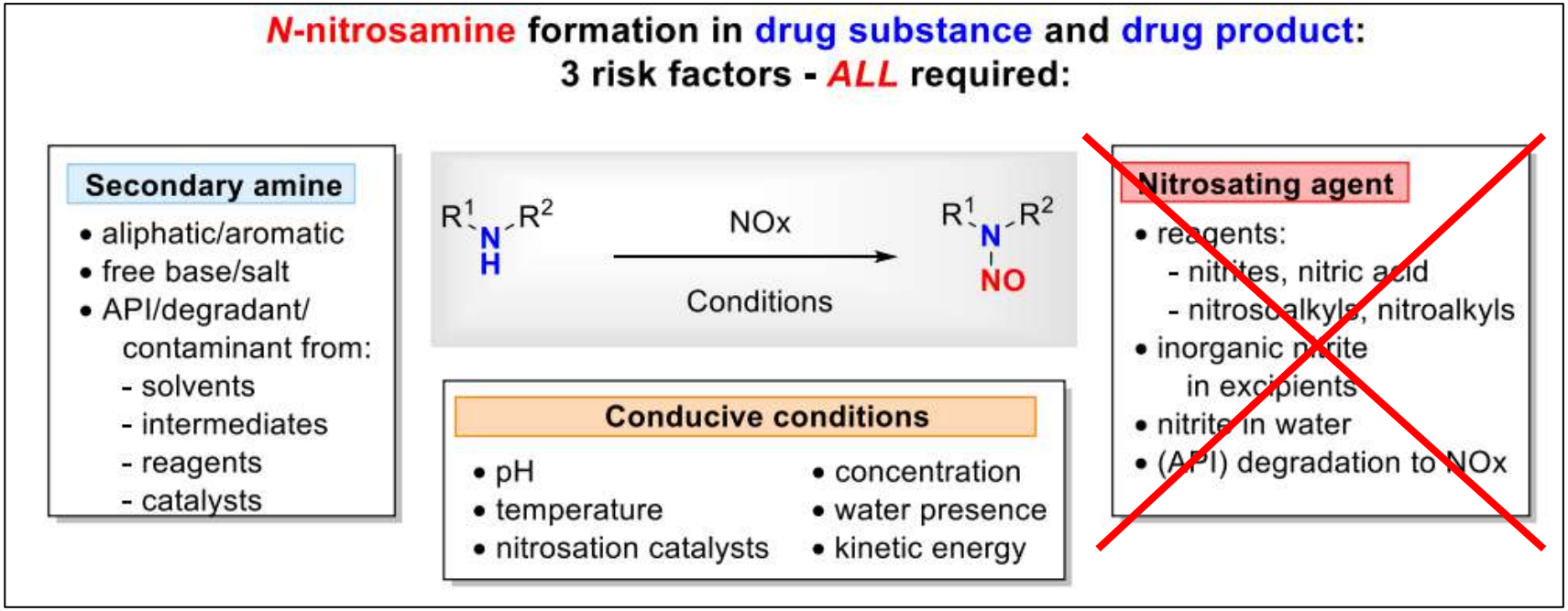
Risk factors



2. Formation of *N*-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies, Cioc et al, Org. Process Res. Dev., 2023, in press.

The Vitic Nitrites Consortium

Generate a comprehensive and robust dataset of the level of nitrites in a broad range of excipients, reagents and solvents to aid in compiling nitrosamine risk assessments for drug products and drug substances.

Vitic nitrites

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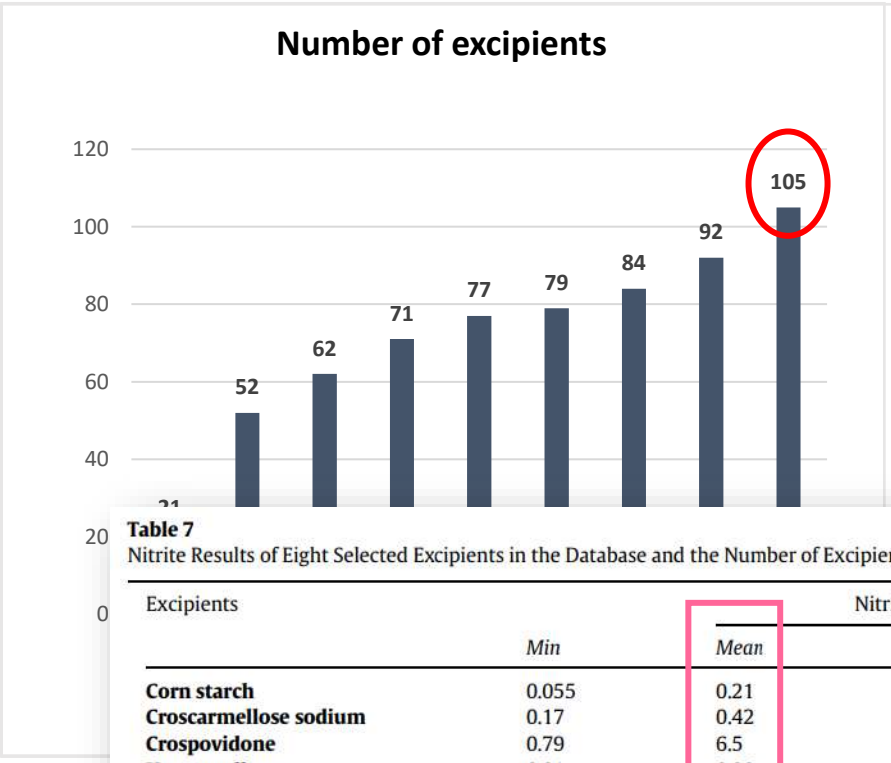
Data sharing initiative



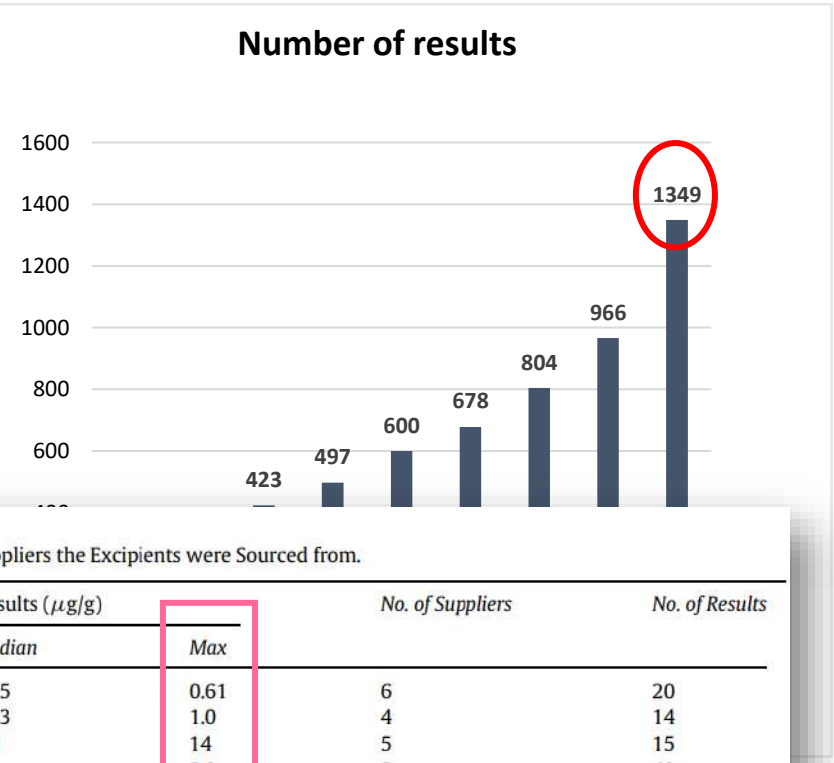


Table 7
Nitrite Results of Eight Selected Excipients in the Database and the Number of Excipient Suppliers the Excipients were Sourced from.

Excipients	Nitrite Results (μg/g)				*No. of Suppliers*	*No. of Results*
	Min	*Mean*	*Median*	*Max*		
Corn starch	0.055	0.21	0.15	0.61	6	20
Croscarmellose sodium	0.17	0.42	0.33	1.0	4	14
Crospovidone	0.79	6.5	8.3	14	5	15
Hypromellose	0.01	0.80	1	5.0	5	49
Lactose monohydrate	0.07	0.54	0.5	1.7	8	34
Magnesium stearate	0.1	2.6	2.4	6.1	9	44
Microcrystalline cellulose	0.04	0.70	0.5	2.4	9	73
Povidone	0.10	0.83	0.5	2.3	5	52

10. A Nitrite Excipient Database: A Useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products, Boetzel et al, J. Pharm. Sci., 2023, 112, 1615-1624.

Calculating nitrosamine formation

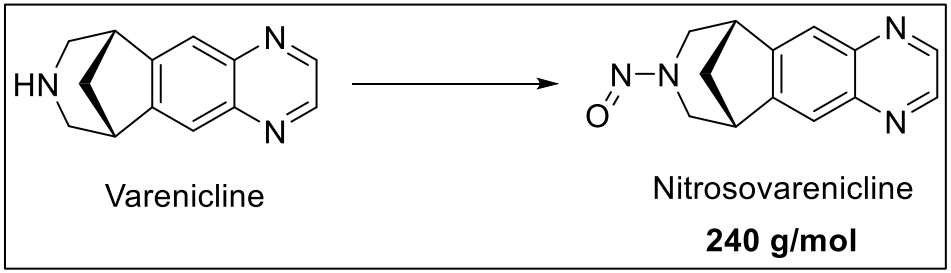
Component of the formulation	Composition in tablet	Mean nitrite	Total nitrite contribution
API	15%	-	-
Microcrystalline cellulose	50%	0.76 ppm	0.38 ppm
Mannitol	22.5%	0.31 ppm	0.07 ppm
Hypromellose	5%	0.6 ppm	0.03 ppm
Crospovidone	3%	6.4 ppm	0.19 ppm
Colloidal silicon dioxide	1%	0.93 ppm	0.009 ppm
Sodium stearyl fumarate	3%	0.28 ppm	0.008 ppm
Magnesium stearate	0.5%	2.1 ppm	0.011 ppm
			0.70 ppm

Calculating nitrosamine content

$$\textit{Nitrosamine [ng/g]} = \frac{\text{Nitrite content [ppm]} * 1000 * \text{MW Nitrosamine [g/mol]}}{\text{MW of nitrite [g/mol]}}$$

Average nitrite level

Vitic nitrites



Safe limit (considering chronic use) = **200 ppm** (6 months @ 2 mg/day)

$$\textit{Nitrosamine [ng/g]} = \frac{\text{0.70 ppm} * 1000 * \mathbf{240}\ \text{[g/mol]}}{46\ \text{[g/mol]}} = \mathbf{3.65\ ppm}$$

10. A Nitrite Excipient Database: A Useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products, Boetzel et al, J. Pharm. Sci., 2023, 112, 1615-1624.

Excipient selection

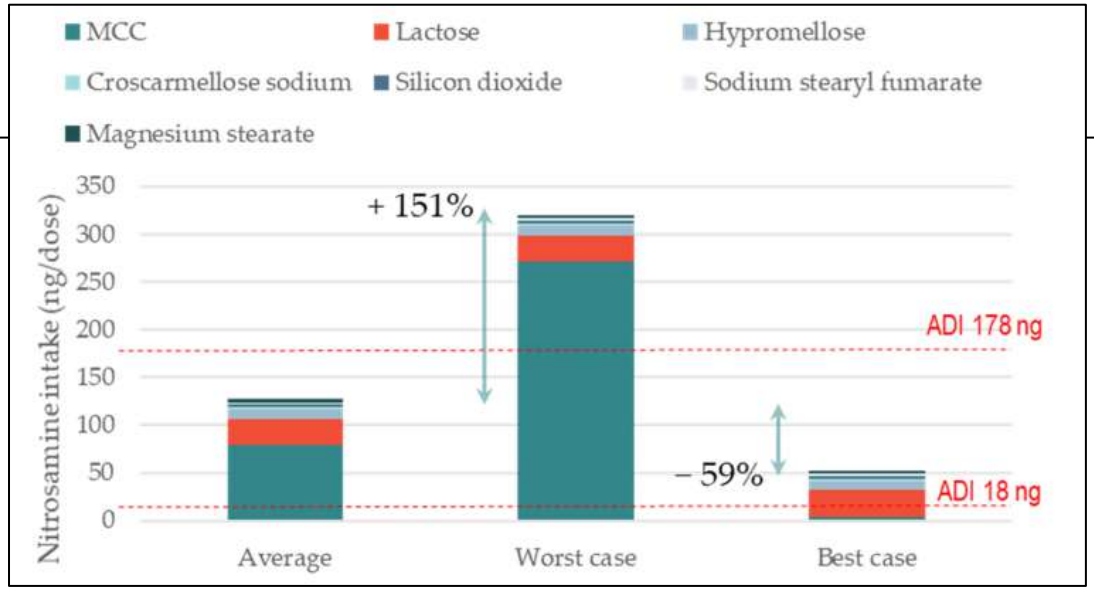
pharmaceutics

MDPI

Article

Modeling the Impact of Excipients Selection on Nitrosamine Formation towards Risk Mitigation

Alberto Berardi *, Maarten Jaspers and Bastiaan H. J. Dickhoff



DFE pharma

Nitrites levels in our excipients are among the lowest in the industry

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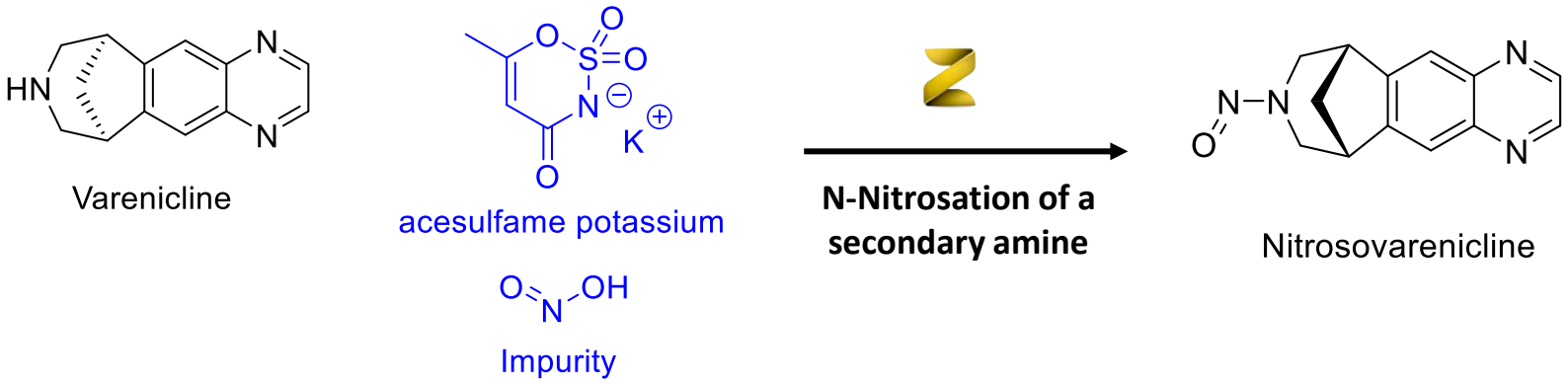
FDA comments

"Regulatory bodies (FDA) indicate **supplier qualification** (e.g., a change of excipient supplier) and **formulation design** (e.g., a change of excipient type) as the main mitigation strategies to reduce nitrosamines, it is key to understand the extent that these strategies can reduce the risk of nitrosamine formation."

12. US FDA. Updates on Possible Mitigation Strategies to Reduce the Risk of Nitrosamine Drug Substance-Related Impurities in Drug Products. *Internet* 2021.

Excipient interaction predictions

- Potential API-excipient interactions can be predicted by Zeneth
- Database of ~350 structures (excipients, and their associated degradants and impurities)
- This can allow a risk-based stability assessment to be done



- Nitrite as an impurity has been added to excipients in Zeneth's excipient database in line with data from the Vitic Nitrites database
- Quantitative data from the Vitic Nitrites database could then be used to calculate the potential amount of nitrosamine in your formulation

Zeneth + Vitic nitrites → Inform and support mitigation strategies

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Conclusions

Nitrosamine formation remains a challenge to assess and mitigate for all organisations involved in the drug development process, including regulators

The *in silico* tool Zeneth can assess the theoretical potential of an API to form a nitrosamine or an NDSRI via a degradation pathway

A database of nitrite levels can be used to understand the impact, and potential amount of nitrosamine formation in your formulation

Zeneth + Vitic nitrites → Inform and support mitigation strategies

Acknowledgments

- Grace Kocks
- Principal Application Scientist
- Project lead for the Vitic Nitrites database
- hello@lhasalimited.org

- Thank you to all Vitic Nitrites consortium members for their data contributions and collaboration.

Thank you to colleagues past and present for the nitrosamine section of Zeneth's knowledge base

References used

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5. In Silico Prediction of Pharmaceutical Degradation Pathways: A Benchmarking Study, Kleinman et al, Mol. Pharm., 2014, 11, 4179-4188.
6. In silico prediction of pharmaceutical degradation pathways: a benchmarking study using the software program Zeneth, Hemingway et al, submitted for publication. ed.
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10. A Nitrite Excipient Database: A Useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products , Boetzel et al, J. Pharm. Sci., 2023, 112, 1615-1624.
11. Modeling the Impact of Excipients Selection on Nitrosamine Formation towards Risk Mitigation, Berardi et al, Pharmaceutics, 2023, 15, 2015.
12. US FDA. Updates on Possible Mitigation Strategies to Reduce the Risk of Nitrosamine Drug Substance-Related Impurities in Drug Products. *Internet* 2021.

Thank you, any questions