Sandoz Development Centre
Early Development

Assessment of a diverse array of nitrite scavengers in solution and solid state: A study of inhibitory effect on the formation of alkyl-aryl and dialkyl *N*-nitrosamine derivatives

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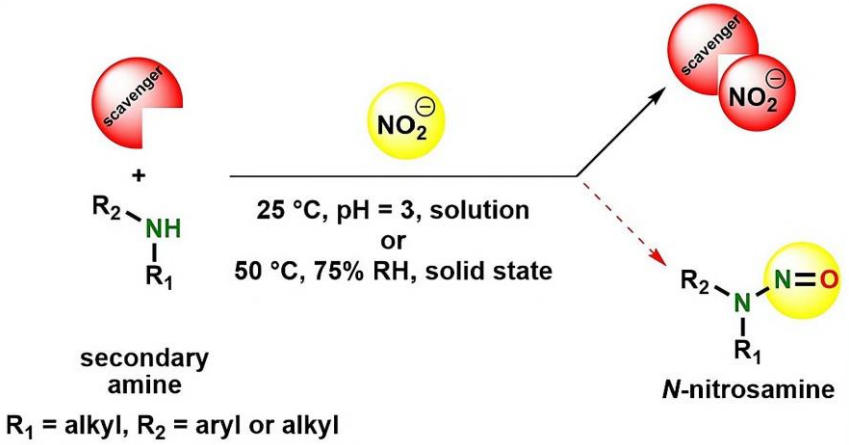
Agenda

- Introduction
- Literature investigation on nitrite scavengers
- Screening of nitrite scavengers' activity
- Evaluation of *N*-nitrosamine formation inhibition in solution
- Evaluation of *N*-nitrosamine formation inhibition in solid state
- Conclusion



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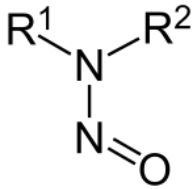
Introduction

Secondary or tertiary amine, **nitrite** and **acidic medium** are key requisites for *N*-nitrosamine formation

Nitrite(s) widespread in **water**, **API**, **excipients**, NO_x may be present in **air**



Food industry: **Antioxidants** (ascorbic acid, α-tocopherol) prevent *N*-nitrosamine formation by **scavenging nitrite** prior to *N*-nitrosation

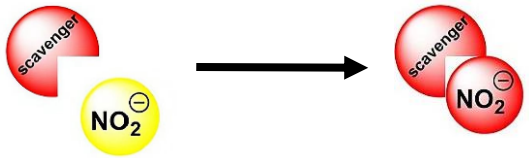
The use of nitrite scavengers suggested by the **FDA** in *Updates on possible mitigation strategies to reduce the risk of nitrosamine drug substance-related impurities in drug products* (November 2021)[1]

Only **one study** published for pharmaceutical samples (Nanda et. al., 2021)[2] at the beginning of our research work

1. https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities
2. *J. Pharm. Sci.* **2021**, 110(12), 3773–3775

Literature investigation

- Various **organic** and **inorganic compounds** react with nitrite(s)
- Desired properties of a nitrite scavenger:
  - **Low toxicological risk** (also for reaction products)
  - Suitable as **Inactive Ingredient for Approved Drug Products** (FDA)
  - **Fast and complete reaction** with nitrite



19 nitrite scavengers chosen for **experimental screening** based on literature data:			
Antioxidants ascorbic acid, sodium ascorbate, ascorbyl palmitate, maltol, phenols – α-tocopherol, resveratrol, propyl gallate, BHA, BHT	**Amino acids** glycine, arginine, lysine, histidine, cysteine	**Inorganic salts** sodium sulfite, ammonium chloride, ammonium sulfate	**Various** PABA, urea

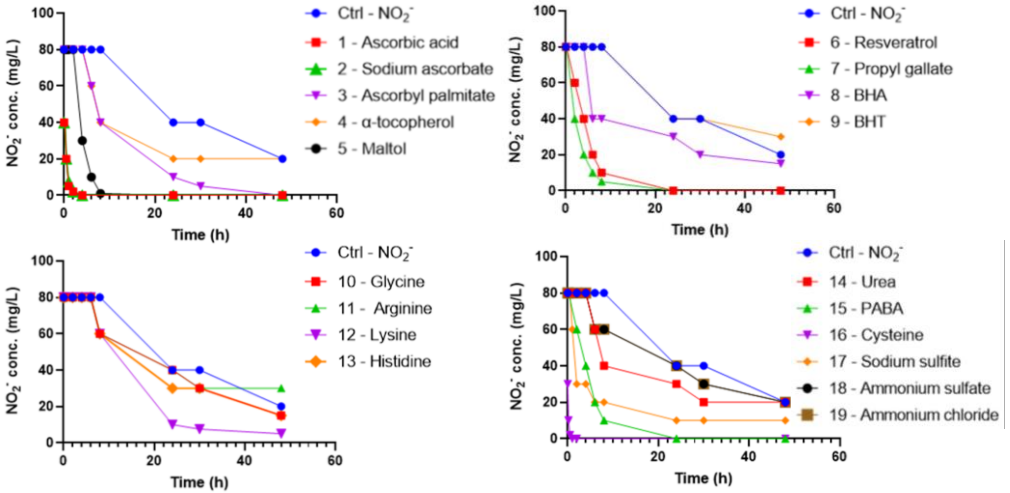
Screening of scavenging activity

4 conditions: pH 3.0/5.0, temp. 20/50 °C

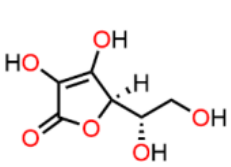
NO_2^- measurement with MQuant® nitrite 2−80 mg/L test strips

Cut-off: consumption of NO_2^- in 20 hours at most conditions

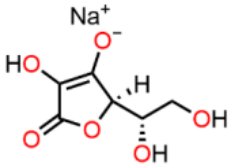
6 scavengers selected for evaluation



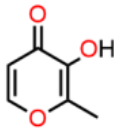
Nitrite:scavenger 1:10 (molar ratio), all reactions carried out at 20 °C in pH 3.0 phosphate buffer.



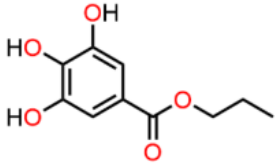
Ascorbic acid
1



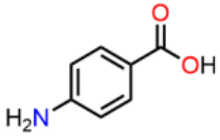
Sodium ascorbate
2



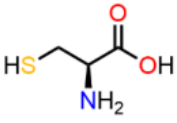
Maltol
5



Propyl gallate
7



PABA
15



L-cysteine
16

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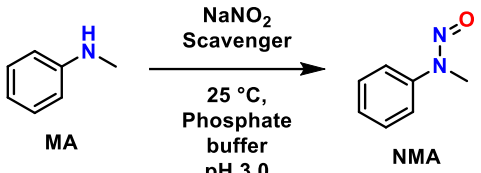
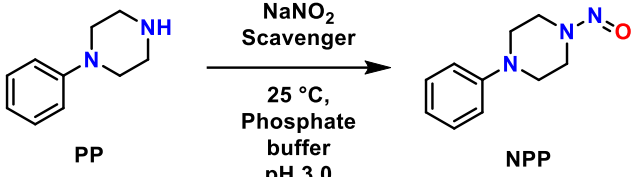
N-nitrosamine inhibition in solution



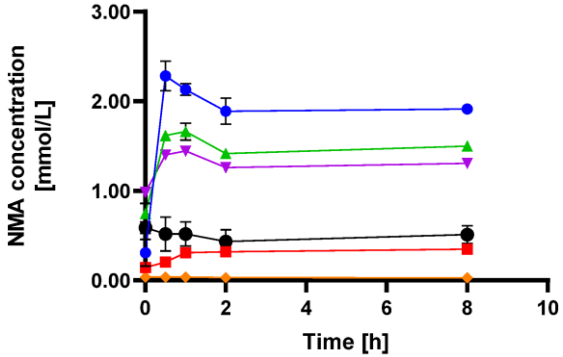
Model APIs:
***N*-methylaniline** (MA)
***N*-phenylpiperazine** (PP)

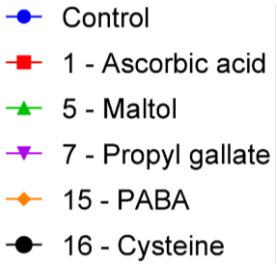


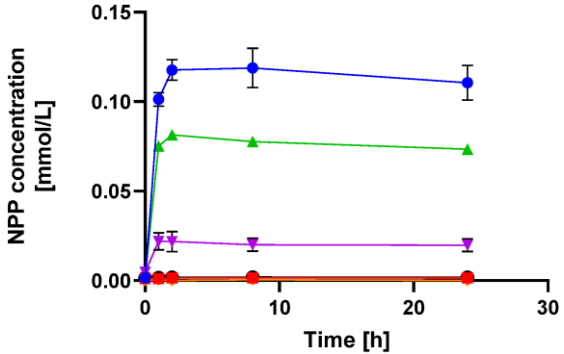
Fast and complete nitrosation of MA
- **PABA** > ascorbic acid ~ cysteine most effective

All scavengers effective, but to a different extent

Lower extent of PP nitrosation (~ 5%)
- **PABA**, **ascorbic acid** & **cysteine** fully inhibited NPP formation







Initial MA or PP concentration was 2 mM, the molar ratio of MA/PP: nitrite: scavenger was 1:1.1:22, and all reactions were carried out at 25 °C in pH 3.0 phosphate buffer.

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N-nitrosamine inhibition in solid state

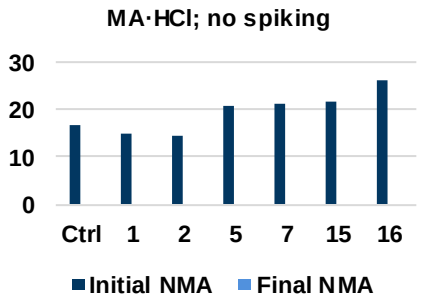
MA and PP formulated in tablets as solid HCl salts (10% API) with scavengers in 10:1 ratio → 28-day **stress test** (50 °C, 75% R.H.)

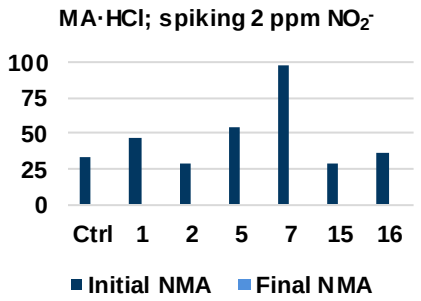
NMA formed already **during tabletting**, **unstable** during stress testing

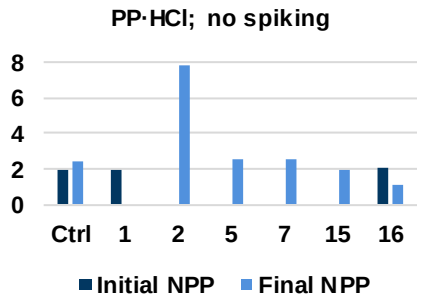
- **Low impact** of scavengers

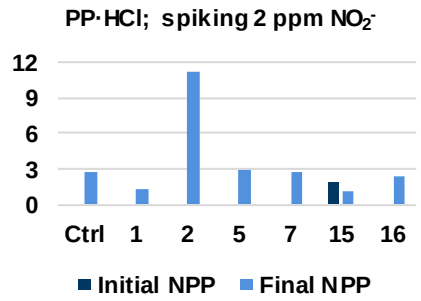
Significant NPP formation during stress test

- **Ascorbic acid**, **PABA**, and ***L*-cysteine** showed some inhibition
- **Na ascorbate** promoted NPP formation!





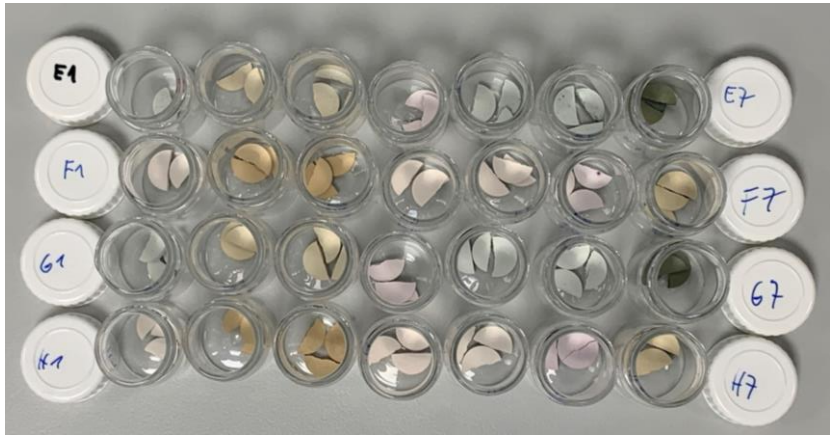




ppm per API; 1: ascorbic acid, 2: sodium ascorbate, 5: maltol, 7: propyl gallate, 15: PABA, 16: *L*-cysteine

Conclusion

- All evaluated nitrite scavengers **lower the formation** of alkyl-alkyl and aryl-alkyl *N*-nitrosamines **in solution**
- **Ascorbic acid**, **cysteine** and ***para*-aminobenzoic acid (PABA)** also effective in **solid state** (tablets)
  - Potential inclusion of PABA to IIADP database
- Nitrite scavenging activity **not linearly correlated** to *N*-nitrosamine formation prevention efficiency



Visual changes of tablets after stress test
(left to right: control, ascorbic acid, sodium ascorbate, cysteine, maltol, propyl gallate, PABA; top to bottom: MA·HCl non-spiked, MA·HCl spiked, PP·HCl non-spiked, PP·HCl spiked)

A **strategy with high potential**, but the choice of scavenger and its amount need to be **evaluated case-by-case** (dosage form, compatibility, stability etc.)

Acknowledgments

- Paper co-authors
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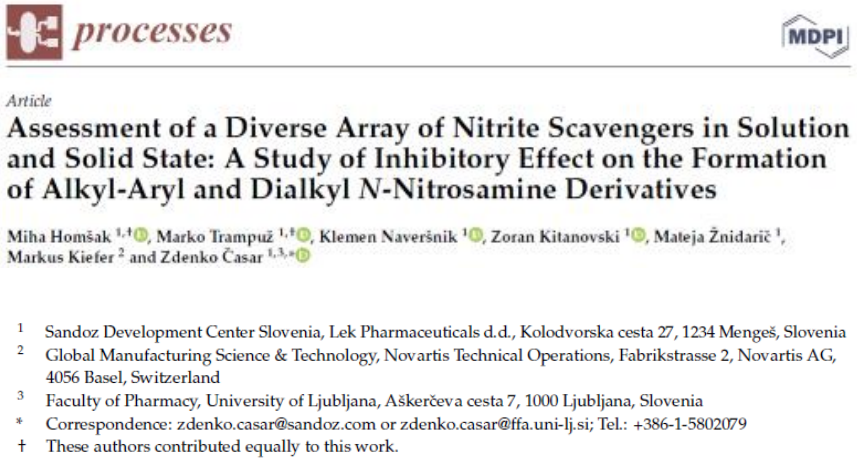
- Food and Drug Administration



processes

MDPI

Article

Assessment of a Diverse Array of Nitrite Scavengers in Solution and Solid State: A Study of Inhibitory Effect on the Formation of Alkyl-Aryl and Dialkyl *N*-Nitrosamine Derivatives

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Thank you

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