The role of Nitrite in Avoiding N-Nitrosodimethylamine Formation in Metformin Pharmaceuticals

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Lhasa Limited Webinar “An Introduction to Nitrite in Excipient Testing to Accelerate Route Cause Investigation of Nitrosamines”

May 17th, 2022
The Healthcare business of Merck KGaA, Darmstadt, Germany operates as EMD Serono in the U.S. and Canada.
• General introduction to nitrosamines
• Role of the amine precursor DMA
• Role of nitrite from excipients
• Analytical challenges
• Complex nitrosamines derived from APIs and their impurities - the next big thing?
General Introduction to Nitrosamines

Nitrous acid

Nitrosation

Cytochrome P450

DNA Repair or Replication

Mutations

Impaired cell cycle Control

Alkylation
Quantification of N-nitrosamines is commonly done by high end mass spectrometry, which is technically complex and uncommon in QC labs.
Nitrosamine Limit \( \left[ \frac{\text{ng}}{\text{g product}} \right] = \frac{\text{AI [ng]} \times \text{DML} \times 1000}{\text{MDD [mg]}} \)

AI  Acceptable Intake per day (18 ng if mutagenicity is unknown)
DML  Drug/Mass Load = Tablet strength / tablet mass
MDD  Maximum Daily Dose

Hypothetical example

\[
\text{Nitrosamine Limit [ppb]} = \frac{18 \text{ ng} \times 0.1 \times 1000}{1000 \text{ mg}} = 1.8 \text{ ppb (ng/g product)}
\]
What is 1 ng/g (ppb)?

Balance weight (stainless steel), 1 g

1 ng/g
1 µg/kg
1 mg/kg
1 g/t
1 g/1000 t

https://weightofstuff.com/14-things-that-weigh-about-1000-tons/
Metformin synthesis

1. DMA
2. 2-cyanoguanidine
3. Metformin
NDMA formation from DMA and Nitrite

\[
\begin{align*}
\text{NO}_2^- & \quad \xleftrightarrow{H^+} \quad \text{HNO}_2 \\
\text{HNO}_2 & \quad \xrightarrow{H^+} \quad \text{H}_2\text{NO}_2^+ \\
\text{Dinitrogen trioxide} & \quad \xrightarrow{H_2O} \quad \text{N}_2\text{O}_3 + \text{H}_2\text{O} \\
\text{DMA} & \quad \xrightarrow{H_2O} \quad \text{NDMA} + \text{HNO}_2 \\
\end{align*}
\]
Metformin API is essentially free from NDMA
NDMA is formed during DP manufacturing
NDMA is formed during DP manufacturing
Nitrite concentration of key excipients

<table>
<thead>
<tr>
<th>HPMC K100M</th>
<th>27% of GXR RM formulation</th>
<th>PVP K30</th>
<th>3.8% of GIR formulation</th>
</tr>
</thead>
</table>

- **Nitrite [µg/g]**
  - Supplier A
  - Supplier C
  - Supplier S

- **Supplier A
  - Supplier B**
Impact of DMA and Nitrite on Glucophage
Impact of DMA and Nitrite on Glucophage XR

Glucophage XR
Impact of LOD and HPMC Supplier

- NDMA [ng/g]
- Loss on drying after granulation [%]

- Glucophage XR RM
- Glucophage XR OF
- HPMC Supplier A

- DMA
  - 10
  - 30
  - 60
  - 90
  - 120

- HPMC supplier
  - Supplier A
  - Supplier D

- Product
  - GXR OF
  - GXR RM
Deriving a DMA limit

(A) Glucophage®

(B) Glucophage® XR

API DMA limit: 141.95 µg/g

API DMA limit: 59.51 µg/g
NOT Preventing NDMA *in situ* formation...

(results from a candidate contract lab vs internal method)

Failure to mitigate *in situ* NDMA formation can lead to artificially high / false positive OOS results.

Nitrosamine *in situ* formation may occur whenever the amine precursor of the nitrosamine analyte is present in the sample (e.g. if the API is a secondary amine).
Poor comparability due to NDMA \textit{in situ} formation in DCM

Residual DMA (API starting material) and nitrite from excipients form NDMA \textit{in situ} in the solvent DCM used for GC-MS analyses.

NDMA \textit{in situ} formation was not observed in 10% MeOH or 50% ACN.

NDMA \textit{in situ} formation can be mitigated by
- LLE starting with water (not feasible for XR products)
- Removal of nitrite and DMA from DCM (Washing)
- Addition of a nitrite scavenger
Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite

Joerg Schlingemann 9, Celine Boucle 9, Sebastian Hickert 9, Laura Bourasseau 8, Matt Walker 9, Caroline Celdran 9, Thibaut Chemarin 9, Celine Puges 9, Matthias Fritzche 9, Judith Keitel 9, Anja Goetsche 9, Mai Seigel 9, Stefan Leicht 9, Brunhilde Guessregen 9, Philipp Reifenberg 9, Stephanie Wetzel 9, Tim Müller 9, Fanny Schooren 9, ... Sandra Masanes 9

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A Nitrite Excipient Database: A Useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products

Ruth Boetzel, Joerg Schlingemann, Sebastian Hickert, Christian Korn, Grace Kocks, Bert Luck, Giorgio Blom, Mark Harrison, Marc Francois, Leonardo Allain, Yongmei Wu, Youssi Bousra

https://doi.org/10.1016/j.phrs.2022.04.016
NDMA analytics in metformin products: Comparison of methods and pitfalls

Matthias Fritzsché, Giorgio Blom, Judith Keitel, Anja Goettzsche, Maié Seegel, Stefan Leicht, Brunhilde Guessreng, Sebastian Hickert, Philipp Reifenberg, Alexandra Cimelli, Romane Baranowski, Emmanuel Desmartin, Elodie Barrau, Mark Harrison, Tony Bristow, Nicholas O’Neill, Annette Kirsch, Phillip Krueger, ..., Joerg Schlingemann
Small molecule drugs in DrugBank, analyzed for the presence of vulnerable amines (*in silico*)

<table>
<thead>
<tr>
<th></th>
<th>SecAmine</th>
<th>TertAmine</th>
<th>SecAmine</th>
<th>SecAmine_Alp</th>
<th>TertAmine_Alp</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11937</td>
<td>1805</td>
<td>2037</td>
<td>1401</td>
<td>1393</td>
<td>2005</td>
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<tr>
<td>100.0%</td>
<td>15.1%</td>
<td>17.1%</td>
<td>11.7%</td>
<td>11.7%</td>
<td>16.8%</td>
</tr>
</tbody>
</table>

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- Tony Bristow
- et al.

Amatsi
- Alexandra Cimelli
- Romane Baranowski
- Emmanuel Desmatin
- Andreas Bathe
- Elodie Barrau
- et al.
Backup slides
In situ nitrosamine formation in DCM isn’t new...


Potential explanations


Nitrite will be present in DCM in protonated form as nitric acid. This can drive its dimerization to form dinitrogen trioxide $\text{N}_2\text{O}_3$, which is the actual nitrosating agent under mildly acidic conditions.
Other pitfalls

- Co-elution of NDMA with dimethylformamide (13C-DMF is isobaric to NDMA)
- Contamination from gloves
- Contamination from sample vials
- NOx related artefacts from sample preparation
- On column nitrosamine generation when using amine mobile phase modifiers
- Contamination during batch sampling with personal care products
- Incorrect assignment of peaks
- Artefactual formation during sample preparation and storage
Preventing \textit{in situ} formation | good comparability of optimized methods

\[
y = 1.07x - 3.11 \quad R^2 = 0.98
\]