Sandoz Development Center
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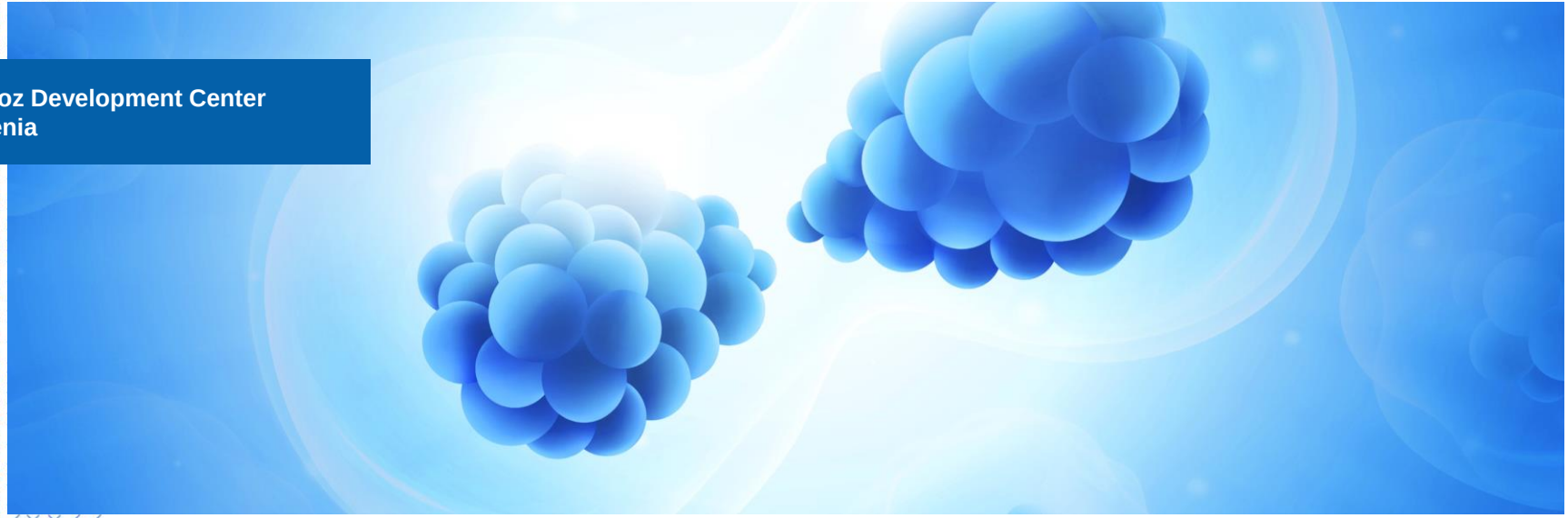

Determination of nitrite in pharmaceutical excipients; air as source for higher nitrite levels.

Rok Grahek

June 15, 2023

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Advancements in Analytical Techniques for Nitrite Detection

Direct method:

- **Ion Chromatography (detection: Conductometric, MS)**

Derivatization

- **UV-Vis Spectroscopy**
- **HPLC (detection: UV, FLD, MS)**
- **CE (detection: UV, ...)**

Advancements in Analytical Techniques for Nitrite Detection

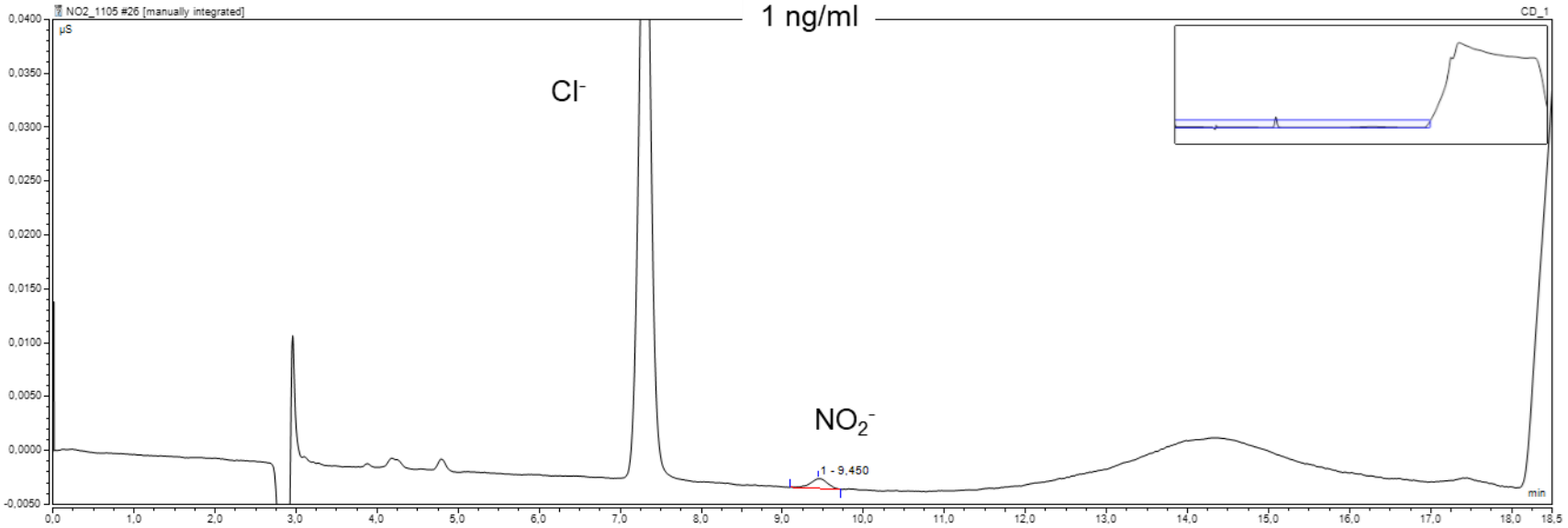
Requirements:

- Robust, easy to use
- Fast, high thruput
- LOQ in excipients 10-50 ppb (0.5-2.5 ng/mL in solution)
- Recovery between 70 and 130%, preferably 90 to 110%
- Affordable equipment

Estimated sensitivity for chromatographic methods regarding detection:
Conductivity ≤ UV < FLD < MS

Advancements in Analytical Techniques for Nitrite Detection

Ion Chromatography (detection: Conductometric)



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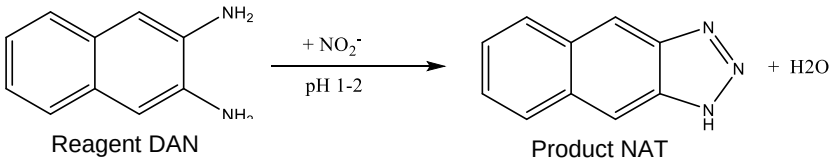
Advancements in Analytical Techniques for Nitrite Detection

Derivatization, two main methods:

- **DAN → NAT**

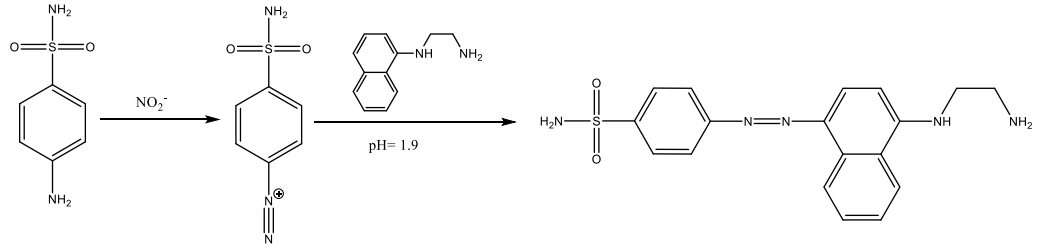


Reagent DAN

Product NAT

FLD ex 375 nm, em 415 nm

- **Griess reagent**

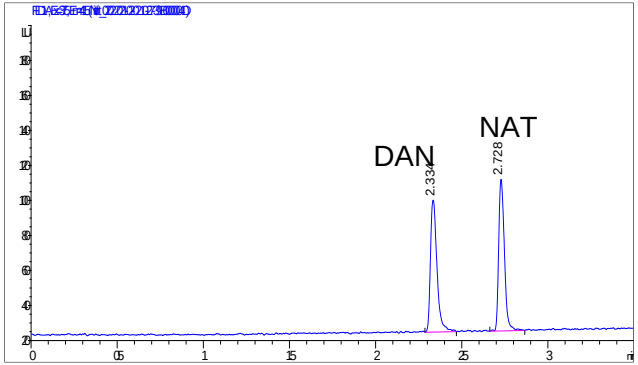


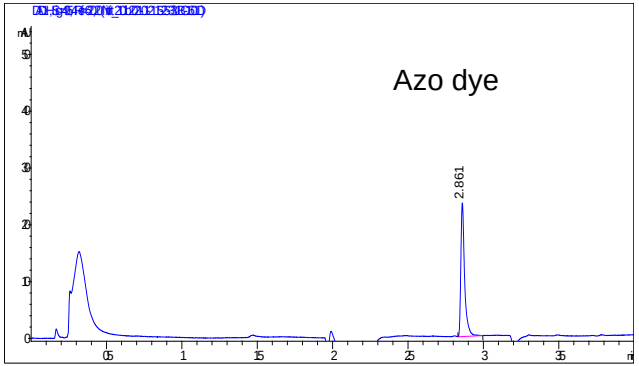
Griess reagent

Pink/purple azo dye

UV 495, 540 nm

Advancements in Analytical Techniques for Nitrite Detection





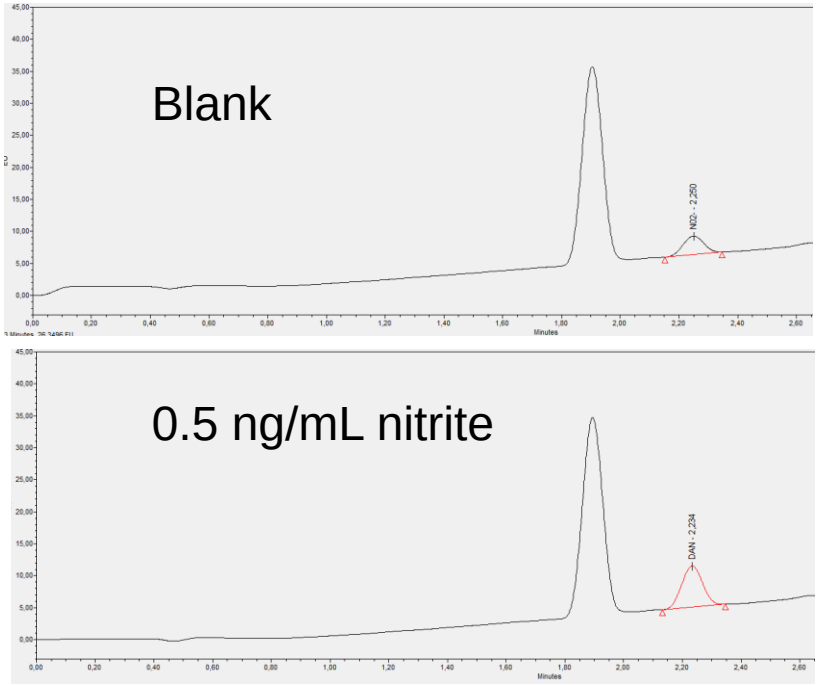
Chromatograms of nitrite standard solution 1.0×10^{-5} mg/mL.

H Li, C J Meininger and G Wu: Rapid determination of nitrite by reversed-phase high-performance liquid chromatography with fluorescence detection. Journal of Chromatography B, 746 (2000), 197-207.

D Tsikas: Analysis of nitrite and nitrate in biological fluids by assay based on the Griess reaction: Appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. Journal of Chromatography B, 851 (2007), 51-70.

Advancements in Analytical Techniques for Nitrite Detection

Technique sensitivity is not the main challenge!



Nitrite is present as contaminant in many chemicals, plastic, filters, glassware...

Nitrite Presence in Common Excipients Used in Pharmaceuticals

The nitrite is determined in excipients of more batches - number of batches in brackets. The levels are informative and does not represent the levels in fresh purchased excipients.

EXCIPIENT	avg Nitrite ppb
Glucose (2)	58
Lactose 80 mesh (3)	57
Lactose 200 mesh (3)	48
Lactose anhydrous (3)	42
Mannitol (3)	103
Aerosil 200 (Sillicon dioxide) (3)	63
Aerosil 380 (Sillicon dioxide)	28

EXCIPIENT	avg Nitrite ppb
Magnesium stearate (3)	2047
Calcium carbonate (2)	3726
Magnesium oxide (3)	6558
Crospovidone (2)	4963
Sodium carboxymethylcellulose (3)	1071
Sodium carboxymethylcellulose (6)	1638

Nitrite Presence in Common Excipients Used in Pharmaceuticals

EXCIPIENT	avg Nitrite ppb
Polyvinylpyrrolidone K30 (3)	330
Polyvinylpyrrolidone K25 (3)	479
Plasdone K90 (PVP) (3)	297
Polyvinyl alcohol (3)	471
MCC 102 (3)	83
MCC 101 (3)	1356
MCC 200 (3)	726
Ethyl cellulose (3)	1453
Klucel EF (Hydroxypropyl Cellulose) (3)	871
Klucel EXF (Hydroxypropyl Cellulose) (3)	601
Klucel GxF (Hydroxypropyl Cellulose) (2)	200
Croscarmellose Sodium (3)	305
Opadry (3)	201

EXCIPIENT	avg Nitrite ppb
Hydroxypropyl Cellulose (3)	498
HPMC HP50 (3)	197
HPMC HP55 (3)	322
HPMC (2)	762
Methocel K100 LV (5)	348
Methocel E15LV (1)	366
Pharmacoat 603 (3)	173
Pharmacoat 606 (3)	148
HPMC-AS-HF	29
TiO2 (3)	211
Talc (3)	545
Crospovidone (3)	114
PEG 6000 (3)	252
Syloid (3)	179

Nitrite Presence in Common Excipients Used in Pharmaceuticals

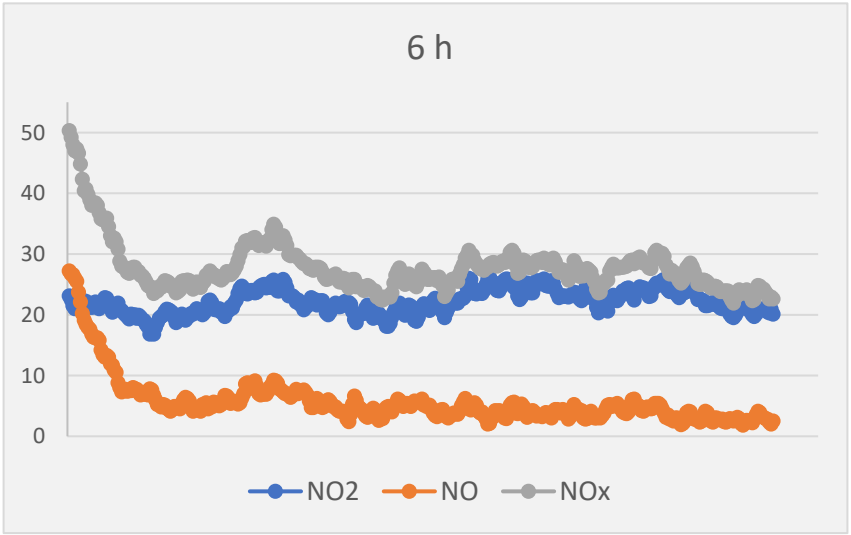
Batch	EXCIPIENT	Nitrite ppb
A	Magnesium stearate	1622
B	Magnesium stearate	4444
C	Magnesium stearate	74
D	Magnesium stearate	951
A	PVP K30	64
B	PVP K30	731
C	PVP K30	195
D	PVP K30	1950
A	MCC 101	2975
B	MCC 101	191
C	MCC 101	902
D	MCC 101	1638

The variation in levels can be high!

Impact of NOx Exposure on Nitrite levels in Excipients

Excipients exposed to air, NOx in air was monitored

NOx in Lab: 6h, avg: NO_2: 22 ppb; NO: 6 ppb; NO_X: 28

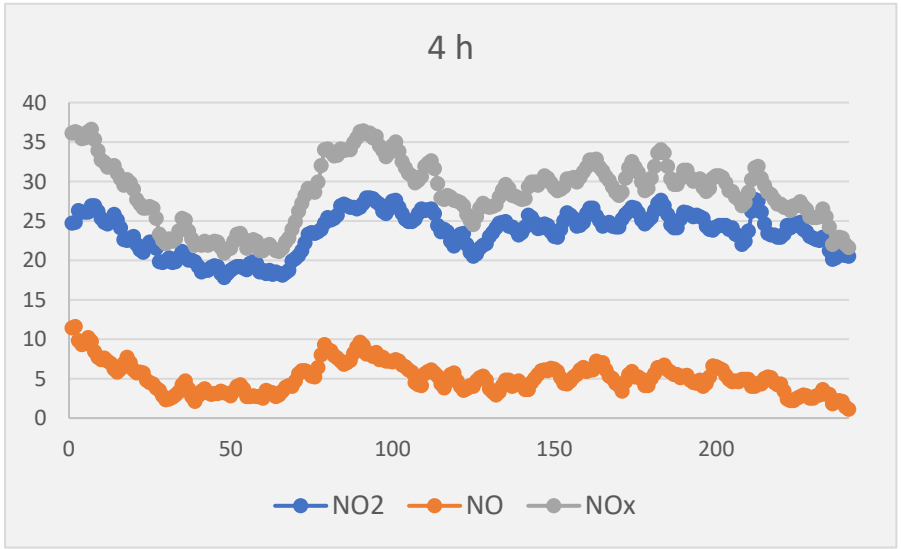


Excipient	Nitrite ppb t=0	Nitrite ppb t=6h
Lactose	< 50	< 50
Starch	< 50	320

Impact of NOx Exposure on Nitrite levels in Excipients

Excipients exposed to air, NOx in air was monitored

NOx in Lab: 4 h; avg NO_2: 24 ppb; NO: 5 ppb; NO_X: 29

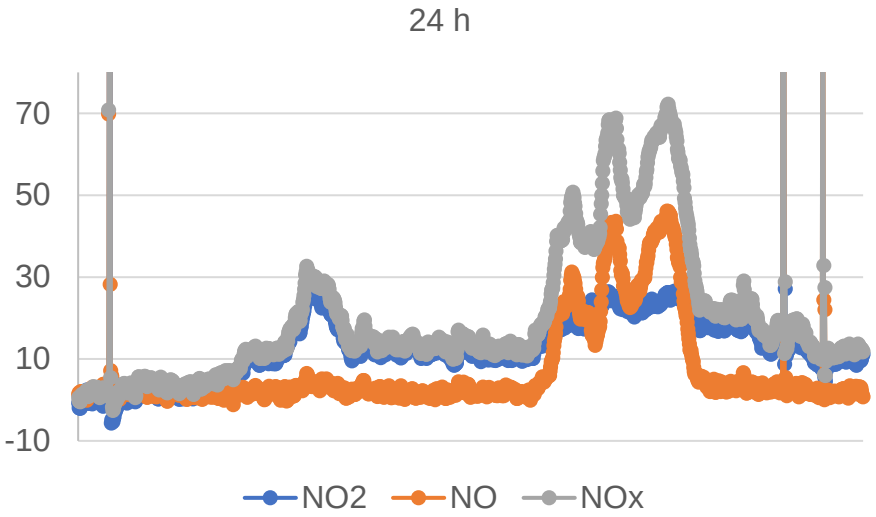


Excipient	ppb t=0	ppb t=4h
Kollidon CL	918	2686
Povidone K30	901	1431

Impact of NOx Exposure on Nitrite levels in Excipients

Excipients exposed to air, NOx in air was monitored

24 h avg: NO_2: 17 ppb; NO: 10 ppb; NO_X: 28



Excipient	ppb t=0	ppb t=24h
Croscarmellose	< 50	138
Lactose Batch A	< 50	< 50
Lactose Batch B	< 50	< 50
Lactose Batch C	< 50	< 50
Starch Batch A	< 50	658
Starch Batch B	< 50	147
Starch Batch C	< 50	507

Case Study: Nitrite Contamination in Tablet Production

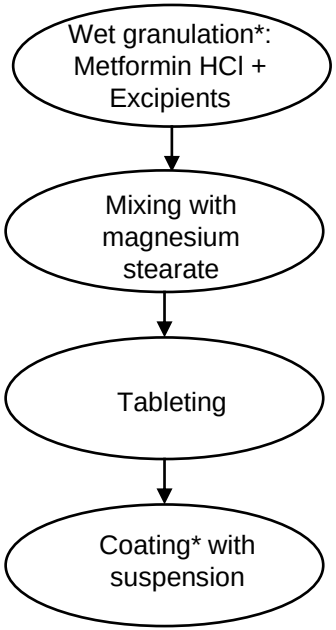
Metformin HCl tablets
Composition, Technology

Typical Composition:

Function	
Tablet core	API
	Binder
Coating	Lubricant
	Film forming agent
	Pigment
	Softner

Excipients can account for about 5% of tablet mass.

Manufacturing process:



*during wet granulation and coating extensive air flows (> 3000 m^3/h) and air temperatures (up to 80 °C) are used

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Case Study: Nitrite Contamination in Tablet Production

Estimated nitrite contribution from excipients in formulation (in ppb).

Component	% in tablet mass	Estimated nitrite contribution in ppb
API	95	70
Excipients	5	30

batch number	Coated/uncoated tablets	NITRITE ppb
A	uncoated tablet	120
A	coated tablet	1045
B	uncoated tablet	113
B	coated tablet	761
C	uncoated tablet	114
C	coated tablet	1004

- Excipients and manufacturing process (wet granulation, coating) are sources of nitrite.
- The main source of nitrite in the tablet is the air used for tablet drying after wet granulation and coating.

Conclusions

- Nitrite determination at ppb level is demanding
- Varaiety of Techniques available
- Nitrite „Background“ is the limiting factor for reaching low ppb levels
- Air with NOx can be the source of nitrite in excipients
- During tablet drying and coating nitrite can be introduced in the FDF

Thanx to my colleagues

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