MEGGLE

# RISK MITIGATION OF NITROSAMINES FORMATION IN DRUG PRODUCTS: Role of Excipients

-NEW-
UPDATE

## How to meet challenges in determining trace levels of nitrite in lactose

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# 1. Introduction

In MEGGLE´s whitepaper "Risk Mitigation of Nitrosamines formation in Drug Products: Role of Excipients" the important issue of Nitrosamine impurities in drug and excipient manufacturing was explained in detail as this topic has become a significant concern for the pharmaceutical industry and health authorities in the last years. The reason for the update is that MEGGLE invested in the development of a new analytical method to even be able to determine trace levels of Nitrite in Lactose. The new analytical method was successfully validated in cooperation with the Technical University Munich (TUM).

As known excipients may contribute to the formation of Nitrosamines through precursor substances present in the excipient (e.g. nitrites, amines). Therefore it is an important achievement that with the new method also trace levels of Nitrite can be determined in MEGGLE´s large Lactose portfolio.

# 2. Nitrites in MEGGLE Excipients – New Method & Results

MEGGLE has been monitoring nitrite content of Lactose regularly since many years. As nitrate content is also important for baby food, MEGGLE implemented a method, specifically designed for milk products, and that is sensitive enough to determine low nitrite content as required for infant milk products (ISO 14673-3:2007- 05). For MEGGLEs pharma grade Lactose it has been found in the past that nitrite is typically not detectable in the Lactose powder (below detection limit of 0.2 ppm). Notably all values are lower than the mean reported for Lactose by Boetzel et al. (2022).

With the new validated, analytical method the determination even of trace levels of Nitrite in Lactose is possible. The new method is based Ion chromatography (IC), this is a liquid-solid chromatographic method used to separate and determine ionic solutes. For IC-Analytics different columns as solid phase and different eluent systems as liquid phase can be selected. The exchange and separation of ions depends on their charge and affinity towards the applied stationary phase (ion exchange column). The separated ions are then detected and quantified using conductivity measurement or UV/VIS spectroscopy. The most common used columns are hydroxide-selective anion-exchange columns, carbonate eluent anion-exchange columns, cation-exchange columns, ion-exclusion columns, amino acid columns and reversed-phase columns. The IC method set-up has been optimized with regards to columns, eluent system, gradient and flow rate as well as the sample preparation to achieve a good resolution for the quantification of Nitrite in the lactose matrix. Based on the method validation with lactose the Limit of Quantification (LOQ) is 0.03 ppm and the Limit of Detection (LOD) is 0.01 ppm.

After the successful validation of the new method, at least 3 samples from different product groups (sieved, milled, agglomerated, spray dried and anhydrous lactose, as well as milled and sieved inhalation grade lactose) have been analyzed. All results showed a value below 0.01 ppm (table 1).

*Table 1. Nitrites levels in MEGGLE Excipients, measured according the new method. LOQ 0.03 ppm, LOD 0.01 ppm.*

| Product Group | Product name | Nitrite Content | Number of Lots Tested |
|---|---|---|---|
| **Sieved Lactose** | SpheroLac® 100 | < 0.01 ppm | 3 |
| **Milled Lactose** | GranuLac® 200, GranuLac® 200 US | < 0.01 ppm | 5 |
| **Agglomerated Lactose** | Tablettose® 70, Tablettose® 80, Tablettose® 100 | < 0.01 ppm | 3 |
| **Spray Dried Lactose** | FlowLac® 90, FlowLac® 100 | < 0.01 ppm | 6 |
| **Anhydrous Lactose** | DuraLac® H | < 0.01 ppm | 3 |
| **Inhalation Grade Lactose sieved** | InhaLac® 120, InhaLac® 230, InhaLac® 251 | < 0.01 ppm | 3 |
| **Inhalation Grade Lactose milled** | InhaLac® 145, InhaLac® 300, InhaLac® 400 | < 0.01 ppm | 3 |

For MEGGLEs pharma grade Lactose portfolio this means that with Nitrite values below 0.01 ppm, they are not detectable, so basically "nitrite free". This lowest amount of nitrite also in MEGGLEs direct compressible (DC) lactose grades (agglomerated, spray-dried and anhydrous lactose) makes them a perfect solution to mitigate/reduce risk of nitrosamines formation for drug product manufacturing.

## 3. IC Application Study for Nitrite Determination

Additionally to the successful validation of the new method with the TUM, MEGGLE also conducted an IC application study together with the company Thermo Fischer Scientific.
In this study several possible IC set-ups for trace level determination of Nitrite have been tested.
One of the main challenges in this study was the interference from the product matrix (matrix effect).
Lactose contains ions from mineral salt and organic acids in the ppm range. They can interfere with the separation and quantification of the target ions, leading to inaccurate results due to co-elution.

For example, the presence of organic acids such as Lactic acid or Citric acid in the matrix can affect the separation and UV detection at the typically used low wavelength for nitrite (210 nm). Similarly, the presence of inorganic and organic anions like Chloride, Phosphate, Citrate or Lactate can interfere with conductivity measurements. To minimize these matrix effects, sample preparation techniques such as in-line dialysis, precipitation or filtration/pre-columns may be used. Selective ion exchange resins and eluents can aid in the separation of the target ions from the interferences in the matrix (Gapper et al., 200; Jireš, J. & Douša, M., 2022, Thermo Scientific Application Note 279).

Several IC system set-ups, like a carbonate system and a gradient hydroxide-system, in combination with conductivity and UV detection have been tested in the Thermo Fischer Scientific application lab. In the test set-up a matrix elimination system was used.

## 3.1 Materials and Methods

In this study lactose solutions were prepared by using lactose with a concentration of 50 g/L. The lower target quantification limit of the method was 20 µg/kg (0.02 ppm) Nitrite related to solid contents, corresponds to 1 µg/l in the test solution. The standard solution contained 1 µg/l Nitrite.

The different columns, eluent and detection systems which were used in this study are listed in table 2.

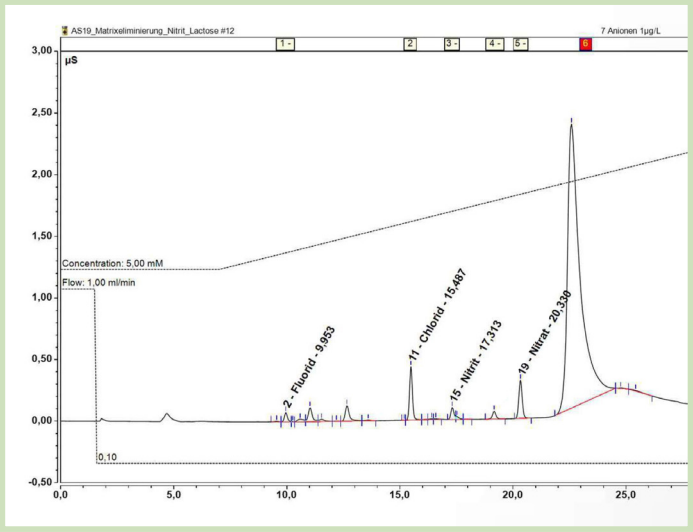
*Table 2. Material and Methods of used IC System. LOQ 0.02-0.05 ppm.*

| **IC System** (Dionex™ Integrion™ and Dionex™ Aquion™) | | |
|---|---|---|
| **Columns** | **Eluent System** | **Detection** |
| IonPac™ AS-14A | Isocratic carbonate system | Conductivity and UV detection (210 nm) |
| IonPac™ AS11-HC, AS-15, AS-18, AS-19 and AS-30 | Gradient hydroxide system | Conductivity and UV detection (210 nm) |

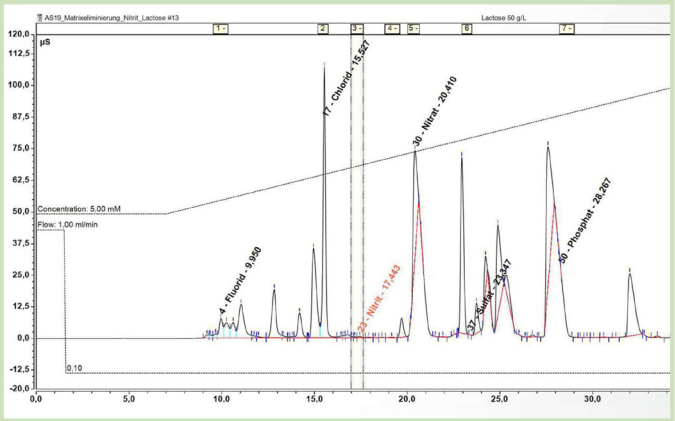
These columns are typically used for analysis of inorganic anions e.g. in water analysis.

Low amounts of nitrite could be quantified with the standard solution (see chromatogram 1). However, when it comes to lactose solution it was not possible to quantify Nitrite reliably at this low level due to co-elution, neither with UV detection nor with conductivity (see chromatograms 2+3).

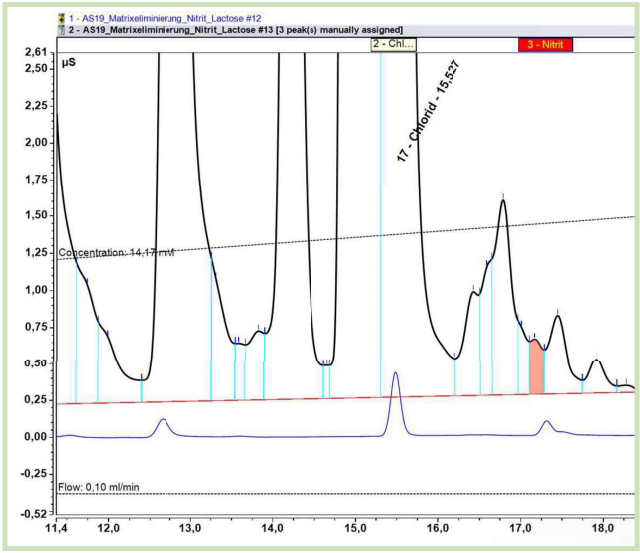
*Chromatogram 1: standard solution (1 µg/L Nitrite) determined with conductivity*



*Chromatogram 2: Lactose solution (50 g/L) determined with conductivity*




*Chromatogram 3: Lactose solution (50 g/L) overlay with Standard Solution*



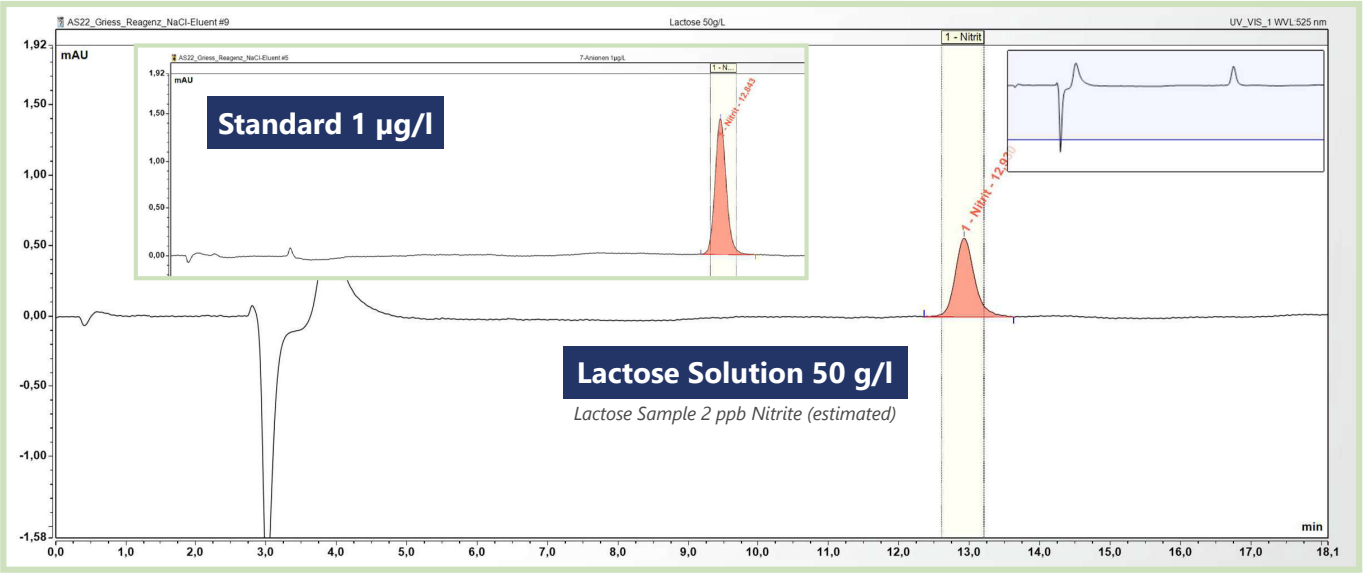

Therefore, as alternative approach the IC system was combined with a post-column Griess derivatization and UV detection at 525 nm.

Also in this approach lactose solutions were prepared by using lactose with a concentration of 50 g/L. The lower target quantification limit of the method was 0.1 µg/l. Nitrite related to solid contents, corresponds to 1 µg/l in the test solution. The standard solution contained 1 µg/l Nitrite.

In this study the Dionex™ Integrion™ with a IonPac™ column AS-22 with NaCl as eluent was combined with a post-column Griess derivatization and UV detection at 525 nm.

With this method 1 µg/l Nitrite could be safely quantified in the lactose solution, no interfering peaks are present (see chromatogram 3). The amount of nitrite for the tested GranuLac® 200 sample was estimated as 2 ppb (0.002 ppm). This is in good agreement with the results of our new validated method (results for our lactose below detection limit of 0.01ppm).

*Chromatogram 4:*
*Comparison of the standard (1µg/L Nitrite) with the Lactose solution (50g/L), determined with IC Plus Post-Column Griess Reaction & UV Detection at 525 nm*




The application study has revealed that the determination of nitrite content at trace levels might be challenging and the product matrix needs to be considered.

The Griess reaction is a well-established method commonly used for detecting nitrites in different samples. This method is based on the selective reaction of nitrite with sulfanilic acid and N-(1-naphthyl) ethylenediamine dihydrochloride under acidic conditions, resulting in a red-violet azo dye that can be measured using spectrophotometry. By comparing the absorbance values of a sample at 540nm to a predetermined standard curve, the concentration of nitrite in the sample can be calculated.
Gapper et al. (2004) reported a high-performance ion-exchange methods incorporating on-line post-column reduction with either cadmium or vanadium, coupled to derivatization with Griess reagent and detection at 540 nm, for dairy products and baby foods. This chromatographic separation of nitrate and nitrite, combined with specific post-column conversion to the chromophoric azo derivative, avoids the potential matrix interference limitations of conventional assays and the inherent disadvantages of other reported chromatographic detection modes.

## 4. Summary & Outlook

In order to minimize the risk of N-Nitrosamine formation in drug products, careful selection of API and excipients in terms of type and amount is crucial. The API manufacturing process and potential impurities or precursors have to be considered primarily. However, also the drug product manufacturing process itself can impact the formation of Nitrosamines. Direct compression is the preferred process as it avoids the use of water and heat. Existing formulations can usually be reformulated accordingly.

MEGGLEs specially designed excipients for direct compression can help you to switch from wet-granulation to direct compression, reduce amount of excipients in the formulation, reduce required compression pressure or decrease the amount of disintegrant needed, thus mitigating the risk of N-Nitrosamine formation.

However, it is important to note that the analytical method used to determine the nitrite content should be carefully reviewed regarding potential matrix interferences. Moreover, pre-tests on actual blends are advisable, as not all solid-state interactions driving N-nitrosamine formation in solid dosage formulations are fully understood yet. Further research is needed to uncover other factors that may affect solid-state reactions, such as local pH gradients, presence of re- active functional groups, counter-ion effects, morphological forms, particle size and surface area of the constituents.

In this paper it was shown that Nitrite determination with IC is possible down to very low levels by using the correct method. However this could be a challenge for lactose due to seen co-elution. Therefore it was not possible to do a reliable quantification of Nitrite at trace level (20 ppm) due to this effect in many different IC test set-ups (6 different columns were tested). The most sensitive test set-up for quantification of Nitrite in a lactose solution was achieved by combining IC with post-column Griess derivatization. The estimated amount of Nitrite for MEGGLEs GranuLac® 200 sample in this test set-up was with 0.002 ppm (2 ppb) extremely low. The reported values for Nitrite in lactose are often significantly higher. With the new validated method MEGGLE is therefore able to show that their complete lactose portfolio is basically “nitrite free”.

**If you would like to learn more about how MEGGLE can help you mitigate the risk associated with Nitrosamines, please contact us.**

**We would like to take the opportunity to thank the TUM and Thermo Fischer Scientific for their engaged, professional and patient collaboration in these studies.**

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