# Lhasa Limited and International Pharmaceutical Excipients Council Europe collaborative meeting report

On 21st March 2024, Lhasa Limited, in partnership with IPEC Europe, hosted a one-day collaborative meeting in Leeds, UK. This report summarises each presentation and includes concluding remarks from Lhasa Limited.

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## Introduction

The one-day collaborative meeting was hosted in Leeds, UK, by Lhasa Limited in partnership with IPEC Europe. The main topics of the meeting included the analysis of nitrite levels in different excipients and the methods used by various manufacturers and users to measure them. There were 50 attendees from 34 organisations located in the US and Europe.

The speakers were from different excipient manufacturers and users, along with representatives from European Directorate for the Quality of Medicines and HealthCare (EDQM) and US Pharmacopeia (USP). They presented their approaches to nitrite testing, the challenges they faced, the results they obtained, and the future perspectives they envisioned. They also discussed the advantages and disadvantages of different nitrite testing methods, such as ion chromatography and HPLC-UV.

A Q&A session was included after each presentation, where the speakers answered questions from the audience and exchanged feedback and suggestions. The meeting concluded with a closing remark from Lhasa Limited, who thanked the speakers and the attendees for their participation and encouraged further collaboration and data sharing on the topic of nitrite in excipient testing.

**Attending organisations**:
Abbott Healthcare Products, Ashland Specialties UK Ltd, AstraZeneca, BASF Corp, Chemische Fabrik Budenheim KG, CHP Carbohydrate Pirna GmbH & Co. KG (JRS), DFE Pharma GmbH & CO. KG, dsm-firmenich, European Directorate for the Quality of Medicines and HealthCare, Eli Lilly and Company, Evonik Operations GmbH, Galenicum Health, German Medicines Manufacturers' Association, Gilead Sciences, GSK, Idorsia Pharmaceuticals, International Flavors & Fragrances (IFF), International Pharmaceutical Excipients Council Europe, Kerry Inc., Krka, d.d., Novo mesto, Lhasa Limited, MEGGLE GmbH & Co. KG, Merck Healthcare KGaA, Novartis Pharma AG, Pfizer Limited, Pharmaceutical Works Polpharma S.A., Roquette, US Pharmacopeia, Sandoz, Sanofi-Aventis Deutschland GmbH, SE Tylose GmbH & Co. KG, Vertex Pharmaceuticals, Zentiva, k.s.

## Session 1

***Nitrosamines in substances and medicinal products – current Ph. Eur. Requirements and EDQM responses***
*Anne Garnier, European Directorate for the Quality of Medicines and HealthCare (EDQM)*

- **Strategy on nitrosamines**: The European Pharmacopoeia (Ph Eur.) has revised its general monographs on substances for pharmaceutical use and pharmaceutical preparations to include a requirement for evaluating the potential risk of nitrosamines in drug products.
- **The analytical methods and task force on nitrosamines**: The Ph Eur. has also developed and validated three analytical methods for the control of seven nitrosamines in active substances and published them in a general text. The methods are currently under revision to include medicinal products as well. The Ph Eur. has also established a task force with the Official Medicines Control Laboratories (OMCL) network and the certification of suitability procedure to provide a coordinated response to the nitrosamine situation.
- **The new working party on excipients**: The Ph Eur. has recently created a new working party on excipients to review the current control of excipients and identify the gaps and challenges. The working party will consult with stakeholders and propose a strategy for improving the quality standards for excipients, especially considering the nitrosamine situation.
- **The upcoming webinar with USP**: The Ph Eur. and the United States Pharmacopeia (USP) will jointly organise a webinar on 18th April 2024 to discuss the harmonisation and discrepancies of the limits and methods for ethylene glycol and diethylene glycol in excipients. The webinar is free and open to anyone interested in the topic.

***Nitrites in excipients – USP perspective***
*Christian Zeine, United States Pharmacopeia (USP)*

- **The perspective on nitrites in excipients:** USP is developing solutions for the determination of nitrites in excipients but does not yet have official standards for them. At this point in time, Christian personally is not expecting that future USP standards will include limits for nitrites in excipients.
- **Strategy for excipient quality:** USP has revised its approach to excipient quality standards since the early 2000s, focusing more on the excipient composition and modernization of official methods.
- **The nitrosamine exchange and analytical hub:** USP has created an online platform for exchanging information and analytical methods on nitrosamines and related topics, where stakeholders can access application notes and discuss with USP scientists and experts.
- **The analytical methods for nitrites:** USP have developed and validated an ion chromatography method for lactose and is working on other methods based on HPLC-Fluorescence detection and HPLC-UV Vis detection for 11 different excipients. They aim to develop a compendial standard for determination of nitrites in at-risk excipients in collaboration with internal and external stakeholders.
- **New risk assessment tool for excipients:** USP is developing a risk assessment tool for excipients that will combine elements from the IPEC questionnaire and the EFPIA decision tree and will be shared on the nitrosamine exchange community.

***The nitrite database - what do we know so far about nitrite in excipients***
*Grace Kocks, Lhasa Limited*

- **Introduction:** Lhasa, a not-for-profit organisation, promotes scientific knowledge and understanding through the development of software solutions to support informed decision making on chemical safety. These solutions are designed by scientists, for scientists, in collaboration with industry stakeholders and regulators. Lhasa has developed and updated our solutions such as Mirabilis, Zeneth, Derek and Sarah Nexus to help its members with nitrosamine risk assessments.
- **The Nitrite Data Sharing Initiative**: Lhasa has created a database of nitrite levels in over 100 excipients, based on data shared by its consortium of pharmaceutical organisations. The database provides an overview of the nitrite contribution from different excipients and helps support with compiling the risk assessment of nitrosamine formation.
- **Nitrite database analysis**: The analysis of the nitrite database showed that the average nitrite content varied among excipients and batches, and that some excipients had higher nitrite levels than others. The analysis also showed that the nitrite contribution was dominated by the highest formula percentage excipients, and that there were differences among excipient vendors.
- **Future directions**: Lhasa and consortium members plan to continue gathering data and expanding the database, as well as extracting more knowledge from the data. The consortium also aims to collaborate with other stakeholders and share its findings with the wider community.

## Session 2

***Excipients Under Scrutiny: Technical Challenges and Strategies for Nitrosamine Reduction***
*Krizia M. Karry, BASF Corp*

- **Excipient control:** Controlling nitrites in excipients is important for reducing nitrosamine risk. Excipient manufactures have improved impurities monitoring programs and some are launching new "low nitrite" or "controlled nitrite" grades. Regardless, at the levels users are requiring for nitrites in excipient (LOD << 100 ppb), identifying and controlling the potential source of nitrites in metric ton scale is a huge challenge. Even smoke outside the manufacturing plant (from something burning), and the lab environment can impact results.
- **Technical strategies:** Technical strategies for reducing nitrosamine risk include using low nitrite excipients, incorporating additional ingredients that limit nitrosamine formation (scavengers/antioxidant), and adjusting manufacturing strategies. A 'no nitrite' world is not realistic so increasing our understanding of the risk is crucial.
- **Supplier selection:** Supplier selection is critical for lowering nitrosamine risk. Switching to excipient manufactures with lower nitrite excipients and ensuring reproducibility of their analytical methods can help reduce risk. Published articles have been shared in which there is too much variability between suppliers for one chemistry (e.g. crospovidones), and some are interpreting this to say the chemistry is bad. Educating the customer to consider reputable and high-quality suppliers is also at the heart of the nitrosamine issue.

***Challenges to determine trace levels of nitrite in lactose***
*Ricarda Leister, MEGGLE GmbH & Co. KG*

- **Nitrite in lactose:** Trace levels of nitrite in lactose can be determined using Ion Chromatography with Conductivity Detection (IC-CD). However, possible co-elution needs to be considered.
- **Optimized IC method**: Different IC setups were tested for nitrite determination in lactose in cooperation with Thermo Fischer Scientific. It has been demonstrated that many commonly used IC method set-ups do not allow a quantification of nitrite at trace level due to co-elution. An optimized method has been successfully validated. The method parameters have been optimized including flow rate, gradient and sample solution preparation, to allow separation and quantification of nitrite peak in lactose matrix. An even lower limit of quantification can be achieved using an IC method with post column Griess derivatization.
- **Conclusions**: Measurement of lactose samples with the new validated method, as well as additional tests with an even more sensitive IC - Griess method, confirmed the extremely low range of nitrite in the tested lactose samples (low ppb level).

***Best practice sharing for nitrite testing – lessons learned within the consortium***
*Sebastian Hickert, Merck Healthcare KGaA*

- **Analytical techniques:** The Lhasa nitrites consortium shares best practices for nitrite testing in excipients within a sub-team. The purpose of this work is to summarize all relevant analytical techniques used by consortium members for nitrite testing in excipients.
- **Method pros & cons:** The most frequently used method according to the survey is Ion Chromatography (IC) with conductivity detection, accounting for over 40% of entries in the database. Pre-column derivatization-based techniques (Griess, DAN) with HPLC separation provide high selectivity and sensitivity. To cover a broad range of excipients, establishing at least two methods, one derivatization-based and one based on IC separation is recommended.
- **Next steps:** The sub-team will continue to share updates on new analytical techniques for testing nitrite and create a living document to share this experience. The nitrite consortium also aims to share the best practice on key analytical techniques in a publication in 2024/2025.

***Managing nitrite impurities – view of an excipient manufacturer***
*Ulrich Reichert, Merck Healthcare KGaA*

- **Nitrite impurities:** Managing nitrite impurities is important for drug products as nitrites are a risk factor for nitrosamine formation so control of nitrites in excipients is key.
- **Nitrite levels and the approach:** Nitrite levels in excipients vary and depend on the supplier and manufacturing process. Merck KGaA have an ongoing project to analyse nitrite levels in excipients with over 400 products in scope. The project tests 3 batches per product (min. 2 batches) and the status as of March 2024 is that about 75% of products are below the quantitation limit (all tested batches), while 25% of products have an effective value in a ppb-range determined (in min one batch).
- **Next steps:** Nitrite information will be presented soon in dossiers. Work is ongoing to develop methods for regular control in products where nitrite concentrations in higher ppb-levels are detected.

## Session 3

***Nitrite consortium comparative testing studies and our learnings***

*Giorgio Blom, AstraZeneca*

- **The purpose and results of the method comparison studies**: The Lhasa Nitrite consortium has conducted two cross-industry studies to compare the nitrosamine testing methods used by different organisation and laboratories and to evaluate the reliability and variability of the data.
- **The challenges and findings of nitrite analysis in different excipients**: The studies revealed that some excipients, such as microcrystalline cellulose and magnesium stearate, posed difficulties in sample preparation and extraction, leading to false positive or overestimated results. Different nitrite analysis procedures developed across the consortium did generate comparable data.
- **The recommendations and implications of the studies:** The studies suggested that standardisation of the analysis procedures is required to minimise the errors and variability in nitrosamine testing, and that care should be taken to avoid interferences and degradation of nitrosamines. For magnesium stearate, the extraction procedure contributed to significant variability of nitrite content reported and we hypothesize sonication can cause overreporting of nitrite content when used as extraction technique. The round robin testing competed has been challenging but a worthwhile task.

***Key excipients and validation challenges***

*Tamas Balogh, GSK*

- **The purpose and scope of the validation guidance update**: The document presents the validation guidance for the analytical procedures to determine nitrite content in pharmaceutical materials, based on the ICH Q2 and the best practices of the nitrite testing. The guidance is intended to share learnings and to ensure the highest quality data for the donation to the Lhasa Nitrite database.
- **The main validation criteria and challenges**: The document outlines the minimum acceptable validation criteria for selectivity, linearity, accuracy, precision, range, limit of quantitation, and solution stability. Some of the challenges faced when validating methods for key excipients such as microcrystalline cellulose, croscarmellose sodium, and magnesium stearate using ion chromatography were also discussed at the Lhasa-IPEC Europe collaborative meeting.
- **The next steps**: The updated consortium validation document is drafted and will be sent out for review to the consortium members, to get agreement on the proposed criteria. The document also aims to be published by the Lhasa nitrite consortium in 2024.

***Parts per billion of nitrite in microcrystalline cellulose by ion chromatography mass spectrometry with isotope labelled internal standard***

*Markus Mintert, International Flavors & Fragrances (IFF)*

- **The motivation and the challenge of the method:** The presentation covered research and methods for measuring nitrite in microcrystalline cellulose (MCC) to address the regulatory and safety concerns regarding nitrosamines. The method had to be sensitive, selective, and suitable for QC labs. Ion chromatography (IC) methods with conductivity and mass spectrometry detection (IC-MS) were developed. The challenge was to overcome the interference and ion suppression caused by other byproducts in the MCC samples.
- **The solution and the validation of the method:** The solution was to use an IC-MS method with isotopically labelled nitrite as an internal standard, which would experience the same ion suppression as the analyte. The method was validated for linearity, accuracy, precision, LOD, and an LOQ of 0.016 ppm. The method showed good performance over a wide range of nitrite concentrations and was able to accurately quantify nitrite in different MCC products. The method was compared to other methods for nitrite analysis, such as IC with conductivity detection and HPLC with derivatization. The IC-MS method was found to be more direct, selective, and simple.
- **The application of the method:** The new IC-MS method was successfully applied for nitrite determination in low nitrite MCC.

## Session 4

***DFE Pharma's road to advanced nitrite level testing in excipients***

*Pauline Janssen, DFE Pharma*

- **DFE Pharma approach:** The presentation described the different methods that DFE Pharma evaluated for quantifying nitrite levels in their excipients. Different methods were used along the way, as the criteria for method selection changed over time. Initially speed was most important, while nowadays costs of testing and detection limits are increasingly important. Evaluated methods have included cadmium reduction and spectrometry, segmented flow analysis, high pressure ion chromatography with conductivity testing, HPLC-UV, and ion chromatography with UV.
- **The results and challenge:** IPEC questionnaires quickly became available for all products based upon existing ISO methods. Over time, different methods were selected to reduce the detection limit of nitrite. IC/PCR/UV detection with a reporting limit of 35 ppb is selected for structural measurement of nitrite levels and reporting on the CoA for microcrystalline cellulose (MCC). Currently ISO segmented flow analyses are performed for structural monitoring of all other products, with typical nitrite levels below the detection limit of 0.1ppm.

***Nitrite analysis in hypromellose: Literature overview and validation of an HPLC method***

*Andreas Sauer, SE Tylose GmbH & Co. KG*

- **Introduction and literature review**: The speaker introduces the topic of nitrite analysis in hypromellose and gives an overview of the existing methods and values reported in the literature.
- **Method selection and optimization**: The speaker explains the criteria for choosing a suitable method for nitrite analysis and describes the challenges and solutions for extracting nitrite from different grades of hypromellose. The HPLC Griess method selected was optimized by using hot water for extraction which is suitable for all substitution and viscosity grades. Robustness evaluation showed little variation of evaporated water during extraction and the influence on the nitrite value is neglectable.
- **Method validation and results**: The HPLC-UV method validation is complete including linearity, precision, accuracy, and robustness with LOQ < 0.03 ppm and LOD <0.01 ppm.
- **Summary and conclusion**: The HPLC-UV method is accurate, precise, rapid, low-cost, and widely applicable for nitrite analysis of hypromellose and other cellulose ether and ester derivatives.

***Challenges in chromatographic analysis of nitrite in PVP-based polymers***

*Volker Neu, BASF Corp*

- **Introduction:** The presentation describes the challenges and solutions for detecting nitrite in natural and synthetic polymers using chromatographic techniques.
- **Ion chromatography with conductivity detection (IC-CD):** IC is a common method for nitrite analysis, but it has limitations such as low sensitivity, matrix interference, and reduced column lifetime. Sample preparation including liquid/liquid extraction enhances sensitivity but cannot be applied to high molecular weight povidones due to phase separation problems.
- **High-performance liquid chromatography (HPLC) with UV detection and Griess reagent:** HPLC is another method that uses a derivatization reaction with Griess reagent to enhance the detection of nitrite. It can achieve lower detection limits than IC. However, controlling the blank value is the major task when targeting low ppb levels of nitrite. E.g. the use of extensively washed plastic labware helps to reduce the blank values. A general observation when analyzing nitrite in PVP based polymers via this method are matrix effects and low recovery rates.
- **Analytical target profile and validation parameters:** The presentation outlined the criteria for selecting an analytical method, such as sensitivity, selectivity, robustness, blank values, minimum matrix interference, validation, and ease of use. It also specified the acceptable range of recovery and precision for nitrosamine analysis.
- **Recommendations and conclusions:** IC method is suitable for low to mid molecular weight povidones with a working range of 2 to 10 ppm. IC with extraction enhances the LOQ to 100 ppb for low and mid MW samples. HPLC with Griess rection is under development. There are indications for LOQ values in the range of 20 ppb for Crospovidones and low to mid MW Povidones. BASF shares the validated analytical methods with its customers, under CDA.

## Closing remarks

The collaborative meeting was organised to discuss with experts, the issue of nitrosamine impurities in drug products and how excipients manufacturers can help mitigate the risk.

A consortium of pharmaceutical companies was formed in 2020 to collaborate on the testing and analysis of nitrite in excipients and to share scientific knowledge and data. A collaboration between the consortium and the excipient manufacturers can help to harmonise the methods and standards for nitrite testing, communicate a consistent message to the regulators and pharmacopoeias, and ensure the quality and safety of drug products.

The Lhasa nitrite consortium is open to collaborative members who are interested in sharing data and expertise on testing for nitrite levels in excipients and invites the excipient manufacturers to contact Lhasa for more information. Thank you to all the speakers, the attendees, and IPEC Europe for their support and sponsorship.

shared **knowledge** • shared **progress**


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