NITROSAMINES RISK MITIGATION:

The critical role of excipients and supplier qualification

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Introduction

The evaluation of nitrosamines in drug products has become a big challenge for many formulators in the pharmaceutical industry. In 2018, the European Medicines Agency (EMA) reported the recall of several drugs containing Valsartan, because of contamination with a nitrosamine impurity. Nitrosamines are potential genotoxic agents and some are classified as probable or possible human carcinogens (Brambilla and Martelli, 2007). The maximum acceptable intake limits for six species of nitrosamines commonly found in pharmaceutical preparations ranges between 26.5-96 ng/day (EMA, 2022a; US FDA, 2021).

Nitrosamine impurities typically originate either from the manufacture of drug substances or are formed in the formulation during shelf-life storage period. Nitrosamines that are formed during drug substance manufacture can typically be purged in subsequent steps, or additional purification steps can be implemented. Nitrosamines that are formed in the final drug product are however not possible to be purged. In case of vulnerable APIs, like secondary amines, nitrosamines can be formed by a reaction with a nitrosating agent, like nitrite under appropriate conditions (for example elevated temperatures, acidic conditions, liquid phase). Figure 1 describes the N-nitrosamine formation for secondary amines upon contact with nitrite. Nitrite is the most important precursor of the ultimate nitrosating agents dinitrogen trioxide (N_2O_3) and nitrosyl ion (NO^+). Nitrates are a secondary focus of attention, given the possible conversion of nitrate to nitrite upon reduction (Boetzel et al., 2022; Lin, 1990; Nanda et al., 2021). Taking into consideration that nitrites are typically only present in low concentration, the build-up of nitrosamines for sensitive APIs can be expected to be proportional to the quantity of nitrite collectively present in the dosage form.

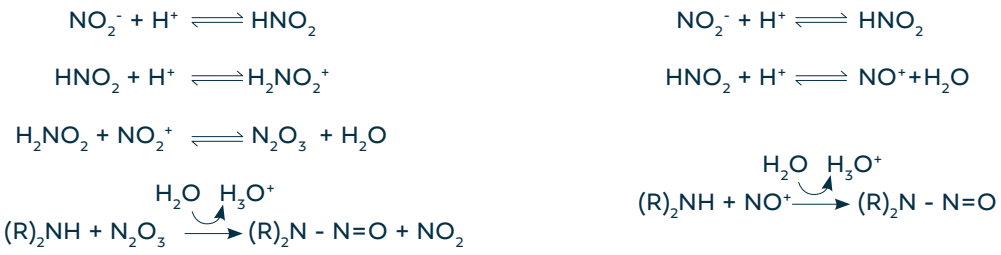

Figure 1. Pathways of N-nitrosamine formation from secondary amines

Market Authorization Holders (MAHs) are now requested by several regulatory authorities to reduce the presence of nitrosamine impurities in their drug products (EMA, 2022b, 2022a; US FDA, 2021). The FDA and the EMA both suggest a three-step mitigation strategy that both API and drug product manufacturers should follow. The first step includes the performance of a risk assessment, the second is the confirmation of the identified risks by testing, and the third consists of reporting changes implemented to prevent and reduce the formation of nitrosamine impurities in drug products.

Risk assessments by the drug product manufacturer should be designed to evaluate the potential sources of nitrosamine formation and contamination during manufacturing of drug products. Excipients are considered as a potential risk factor during the drug product risk assessment; the risk of the presence of nitrosamines is very low. However, many excipients contain traces of nitrites that can ultimately result in formation of nitrosamines under specific conditions within the drug product. Excipient suppliers should provide information about excipients to assist the MAH in their evaluation of the risk of the presence of nitrosamine impurities in the final drug product (IPEC Federation, 2022).

Multiple mitigation strategies are described in the guidance documents of the FDA and the EMA, all aiming to reduce nitrosamines formation (EMA, 2022b; US FDA, 2021). Two key strategies include formulation design and supplier qualification, although it is also encouraged to consider innovative mitigation strategies.

Formulation design

All formulation ingredients, including excipients can contain nitrosating precursor substances, like nitrites, which could contribute to the formation of nitrosamines in drug products. Reformulation towards different excipients with lower nitrite content could therefore be beneficial to suppress the formation of nitrosamines. Another reformulation strategy could include the addition of antioxidants, such as ascorbic acid or alpha-tocopherol, to inhibit the reaction (Mergens, 1982; Nanda et al., 2021; Ziebarth and Scheunig, 1976). Reformulation strategies with addition of inhibitors should however be considered with care, as inhibitors are typically highly reactive species which could interact with the API or other chemical entities within the dosage form. It is also thought that the rate of nitrosamine formation can be inhibited by increasing the pH of the micro-environment, as the nitrosamine formation reaction is catalyzed by acidic conditions (Ziebarth and Scheunig, 1976). The incorporation of pH-modifiers, like sodium carbonate, could therefore reduce the formation of nitrosamines as well. Also in this case, care should be taken that pH-modifiers do not interfere with the stability of the API.

Supplier qualification

Supplier qualification is a mitigation strategy that is also described in the FDA guidance and that might have less impact on the registration dossier of the drug product. This is based on the observation that nitrate and nitrite levels can significantly differ across excipient lots and suppliers (Boetzel et al., 2022). Many excipient suppliers provide nitrite information based upon risk assessment only. Alternatively, when data is available, nitrite content might be measured with analytical methods that are poorly sensitive (i.e. unable to detect low levels of nitrites), thus, making differentiation between suppliers difficult.

Benchmarking excipient types and suppliers on nitrites levels

Whether MAHs opt for the strategy of reformulation with different excipients or supplier qualification, the scientific literature can serve as a valuable tool to appreciate the extent of excipient-to-excipient, supplier-to-supplier and batch-to-batch variability in nitrite levels. In an early investigation, Wu et al. screened the levels of nitrites and nitrates of various fillers, superdisintegrants, binders, lubricants and glidants (Wu et al., 2011). Results showed that these impurities are omnipresent in excipients, although the levels seemed highly dependent on the both the excipient type and the supplier. It was suggested that this is due to minor manufacturing differences, as processing water, acid titration steps, bleaching and oxidation of air during drying, which could all contribute to the presence of these impurities in the excipients. More recently, Lhasa Limited has built a “Nitrites in Excipients” database and some of the results are shared in the article of Boetzel et al. The main findings were that 1) both average and variance in nitrites levels are highly different among excipients; and 2) with the same excipient type, different suppliers show major differences in values (Boetzel et al., 2022).

Excipients for which reformulation (i.e. changing to an alternative excipient) or supplier qualification (i.e. changing to an alternative supplier) is more important are those that are present in large percentage in the formulation, as is the case of fillers. The contribution of impurities in these excipients is relatively higher to that of low-dosed ingredients such as disintegrants, glidants and lubricants. However, even for the low-dosed excipients, selection shall also be considered in some specific cases. For example, the superdisintegrant crospovidone showed a mean level of nitrites which was 9 to 12-fold higher than that of the fillers microcrystalline cellulose and lactose. Therefore, the overall contribution of nitrites coming from crospovidone shall be considered high, despite its relatively low concentration in the formulation. Replacement of crospovidone with croscarmellose sodium (15-fold lower mean value of nitrite) could be a viable option for formulations containing sensitive APIs (Boetzel et al., 2022).

Within the same excipient type, different suppliers provide excipients with distinctively different levels of impurities. Table 1 shows the median nitrite levels of eight suppliers of MCC, as reported by Boetzel et al. It should be noted that the number of samples tested is different for each supplier. For more details readers can refer to the original article (Boetzel et al., 2022). The median nitrite level of different suppliers can vary substantially, up to a factor of more than 16.

Table 1. Nitrite levels (median) in MCC from different vendors (Boetzel et al., 2022). ppm = µg/g

	Supplier							
	#1	#2	#3	#4	#5	#6	#7	#8
Nitrite	1 ppm	0.1 ppm	1 ppm	0.2 ppm	1 ppm	1.2 ppm	0.2 ppm	1.6 ppm

Nitrites and nitrates in DFE Pharma excipients

DFE Pharma is one supplier of excipients that from the early beginning has tried to understand the issue of nitrosamines formation. The basis of their expertise relates to the knowledge gained by the mother company FrieslandCampina. This dairy company already had experience in nitrosating agents, because of the strict regulations regarding contaminants in (infant) food (European Union, 2006). The filled IPEC Europe questionnaire was the first step of DFE Pharma to support MAHs with their nitrosamine risk assessment. This questionnaire includes a matrix to consider the structure and the origin of the excipient as a first indication of risk. Factors considered were the presence of nitrosating agents in the production process, the type of water that is used, the use of recycled/recovered solvents, the presence of amines, amides, primary amines or ammonium salts and the multipurpose use of the equipment. It was concluded that the probability that nitrosamines and nitrosating agents are present in their excipients is very low. Analytical data confirmed that nitrosamine, nitrate and nitrite content were below the detection limits.

Given that the initial method of quantification was not sensitive enough to detect the low levels of nitrites and nitrates present in the excipients, a more sensitive method was identified (ISO-14673-2: 2004 (E): segmented flow analysis). This method is validated for milk and milk products and not specifically for excipients. Table 2 shows the content of nitrites and nitrates in the excipients of DFE Pharma.

Table 2. Nitrites and nitrate levels in excipients from DFE Pharma as measured by the ISO-14673-2: 2004 (E): segmented flow analysis. Limit of detection (LOD) and limit of quantification (LOQ) are indicated. SSG = sodium starch glycolate and CCS = croscarmellose sodium

Product class	Brand name	Nitrate (LOD=0.1ppm; LOQ=0.3ppm)	Nitrite (LOD= 0.03ppm; LOQ= 0.1ppm)
MCC	Pharmacel®	0.8 ppm	<0.1 ppm
Milled and sieved lactose	Pharmatose®	0.7 ppm	<0.1 ppm
	Lactochem®	1.4 ppm	<0.1 ppm
Direct compression lactose	SuperTab®	0.7 ppm	<0.1 ppm
	SuperTab® NZ	7.7 ppm	0.7 ppm
Inhalation lactose	Respitose®	0.7 ppm	<0.1 ppm
Superdisintegrants	Primojel® (SSG)	2.0 ppm	<0.1 ppm
	Primellose® (CCS)	0.6 ppm	<0.1 ppm
Starches	Solani Amylum	0.5 ppm	<0.1 ppm
	Prejel	0.6 ppm	<0.1 ppm
Stabilizers	BioHale® sucrose	0.4 ppm	<0.1 ppm
	BioHale® trehalose	0.4 ppm	<0.1 ppm

Values of nitrites are typically lower than the LOQ of 0.1 ppm. The only limitation of this approach is the fact that most products had levels below the LOQ of the detection method. Therefore, the value of <0.1 ppm nitrites indicated for most products is an overestimated worst-case scenario and it is unknown how low are the exact values. Precise data will be collected once a more sensitive method of quantification will be available for testing. Despite the different quantification methods, a comparison of the obtained data with those reported in the publication of Boetzel et al. can be attempted. It appears that for MCC, lactose and superdisintegrants, DFE Pharma belongs to the suppliers with the lowest levels of nitrites. Only the direct compression lactose produced in Kapuni, New Zealand, has values slightly higher than 0.54 ppm, which is reported as mean value for lactose across different suppliers (Boetzel et al., 2022).

Future outlook

The regulatory health authorities and the pharmaceutical industry together are taking important steps to reduce the risk of nitrosamine exposure to patients. Excipient suppliers have a crucial role in the pharmaceutical supply chain and have the responsibility to control the content of nitrosating agents in their products. Ideally, excipient suppliers should be able to supply high quality and facilitate safe use of their excipients by maintaining low nitrite levels, combined with an implemented control strategy to ensure these low levels are obtained consistently. A current challenge to implement this approach, however, is the capability of analytical methods to detect low levels of nitrites (Wang et al., 2017). The availability of analytical methods with quantification levels down to part-per-billion (ppb) levels would be desirable. For high-risk formulations, improved analytical methods could lead to nitrite specification limits agreed between excipients suppliers and drug product manufacturers. The reasonable expectation is that to have excipients low on nitrites and nitrates. Claims of nitrites and nitrates free excipients are however unrealistic because minimal traces in excipients are inherently present in the raw materials used to manufacture the excipients and cannot thus be removed. Altogether, the proposed steps can support end product manufacturers to reduce nitrosamine impurities in their drug products – allowing us to move jointly towards a healthier world.

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To learn more about how DFE Pharma can help you to mitigate nitrosamines related risk, please contact **Alberto Berardi: *alberto.berardi@dfepharma.com*.**

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