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Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite

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ABSTRACT

Since late 2019, concerns regarding trace levels of the probable human carcinogen N-dimethylnitrosamine (NDMA) in Metformin-containing pharmaceuticals have been an issue if they exceeded the maximum allowable intake of 96 ng/day for a medicine with long-term intake. Here, we report results from an extensive analysis of NDMA content along the active pharmaceutical ingredient (API) manufacturing process as well as two different drug product manufacturing processes. Our findings confirm that Metformin API is not a significant source of NDMA found in Metformin pharmaceuticals and that NDMA is created at those steps of the drug product manufacturing that introduce heat and nitrite. We demonstrate that reduction of nitrite from excipients is an effective means to reduce NDMA in the drug product. Limiting residual dimethylamine in the API has proven to be another important factor for NDMA control as dimethylamine leads to formation of NDMA in the drug products. Furthermore, analysis of historical batches of drug products has shown that NDMA may increase during storage, but the levels reached were not shelf-life limiting for the products under study.

1. Introduction

100 years after its first synthesis in 1922 (Werner and Bell, 1922), Metformin is today the most widely prescribed first-line oral glucose-lowering agent for the treatment of type 2 diabetes mellitus (Graham et al., 2011). In 2011, Metformin was included in WHO's essential medicines list (Bailey, 2017).

Metformin hydrochloride is synthesized from the starting materials dimethylamine (DMA) hydrochloride and 2-cyanoguanidine. For this reason, even the finished API contains small amounts of DMA in hydrochloride form, which may react under suitable conditions with nitrosating agents to form the *N*-nitrosamine NDMA (Fig. 1). In May 2020, the FDA informed about NDMA exceeding the acceptable intake limit in several lots of an extended-release formulation of Metformin (FDA, 2020). Since then, there have been>258 recalls of Metformin containing products in the US alone, some limited to individual batches, others extending to all product batches on the market (FDA, 2021). Meanwhile, EMA has requested marketing authorization holders to test their Metformin containing products for NDMA before they are released to the market (EMA, 2020a), until completion of the root cause

Nonstandard Abbreviations: AI, Acceptable Intake; API, Active Pharmaceutical Ingredient; CCM, Crude Crystallization Mix; CPV, Continuous/Continued Process Verification; CWM, Crude Wet Metformin; DCM, Dichloromethane; DMA, Dimethylamine; DP, Drug Product; EDQM, European Directorate for the Quality of Medicines; EMA, European Medicines Agency; FCM, Finished Crystallization Mix; FDA, U.S. Food and Drug Administration; GIR, Glucophage® Immediate Release Tablets; GXR, Glucophage® Extended Release Tablets; HPMC, Hydroxypropymethylcellulose; IC, Ion Chromatography; IR, Immediate Release; MMA, Monomethylamine; MDD, Maximum Daily Dose; NDEA-d4, N-nitrosodiethylamine-d4 (deuterated); NDMA, N-nitrosodimethylamine; NDMA-d6, N-nitrosodimethylamine-d6 (deuterated); NMT, Not more than; OF, Old Formulation; Ph.Eur., European Pharmacopoeia; PVC, Polyvinylchloride; PVDC, Polyvinylidenchloride; PVP, Polyvinylpyrrolidone; RM, Reduced Mass Formulation; RSD, Residual Standard Deviation; SST, System Suitability Test; TMA, Trimethylamine; UCL, Upper Control Limit; USL, Upper Specification Limit; WFM, Wet Finished Metformin; WHO, World Health Organization; XR, Extended Release.

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investigation and implementation of a NDMA control strategy ensuring that NDMA will consistently be below established limits.

We have performed an extensive analysis of NDMA content along the API manufacturing process as well as for two different drug product manufacturing processes (Glucophage® & Glucophage® XR) to identify the process steps during which NDMA is either created or depleted. Furthermore, we have performed a data mining exercise based on NDMA analysis of>2000 historical batches, to identify key factors impacting the extent of NDMA formation in the drug product. Finally, we have monitored NDMA formation in different packaging systems under stress, accelerated and long-term conditions. Here we describe our root cause investigation for the presence of NDMA in Metformin drug products and our control strategy. Understanding the factors influencing NDMA content in drug products has allowed us to take measures that ensure production of Metformin drug products with NDMA levels consistently well below acceptable intake limits.

NDMA is formed by the reaction of DMA with a suitable NO donor. Secondary amines such as DMA are considered to be among the most reactive amines for forming nitrosamines, particularly under acidic conditions (Ashworth et al., 2020). Nevertheless, this reaction can also occur in basic medium under specific conditions such as photochemical activation or in the presence of catalysts (Beard and Swager, 2021; López-Rodríguez et al., 2020). The formation of NDMA from DMA takes place at variable reaction rates depending on pH. This complicates risk assessment as pH must be considered as a parameter for variability, too. The API of Glucophage® products is Metformin hydrochloride. Residual DMA (Ph.Eur. impurity F in Metformin), as hydrochloride, is present in the API, with a specification of NMT 0.05%. Traces of nitrite or other species with nitrosation potential are ubiquitous and can be impurities of excipients used in the drug product manufacturing process. The combination of DMA and nitrosating agents can lead to the formation of NDMA as shown in Fig. 1.

2. Materials and methods

2.1. Source of materials

Samples of drug substance, drug products, and excipients were obtained from the manufacturing sites' repositories of reference materials. Metformin API and drug products were manufactured by Merck Santé S. A.S. (Lyon, France (an affiliate of Merck KGaA, Darmstadt, Germany) as described below, whereas excipients were bought from external suppliers. Supplier names can't be disclosed for reasons of confidentiality. During the API and drug product manufacturing process, samples were taken at the indicated steps as described in Fig. 3 and Fig. 4 for the API and Fig. 2 for the drug products.

2.1.1. Metformin hydrochloride API manufacturing

Metformin hydrochloride is manufactured following a two-step process. In step 1, Metformin hydrochloride is synthesized from DMA hydrochloride and cyanoguanidine, followed by a first crystallization. In step 2, Metformin hydrochloride is purified by recrystallization, centrifugation, and drying. For some drug product processes, the API is blended with 0.5% magnesium stearate to avoid agglutination, yielding the so-called API premix. API and API premix are manufactured at two production sites C and M. For details refer to section 3.1.

2.1.2. Metformin drug product manufacturing

The composition of Glucophage® and Glucophage® XR tablets is summarized in Table 1 together with results from nitrite determinations in the API and respective excipients. To identify steps in the drug product manufacturing processes that promote the formation of NDMA, samples were taken as indicated in Fig. 2 from several batches manufactured at full commercial scale at the production sites SE and MO. There are two formulation variants of Glucophage® XR, the initial "old formulation" (OF) and the revised "reduced mass" formulation (RM). All samples taken from the manufacturing of Glucophage® XR were from the RM formulation.

2.1.2.1. Glucophage®. Glucophage® tablets were manufactured following a standard process, consisting of wet granulation of Metformin using PVP aqueous-based binder solution in a high shear granulator, fluid bed drying, sieving, lubrication by addition of magnesium stearate, tableting and film-coating (Fig. 2A).

2.1.2.2. Glucophage® XR. Glucophage® XR tablets (RM) were manufactured following a standard process, consisting of wet granulation of the mixture of Metformin premix and the carmellose sodium with purified water in a high shear granulator, fluid bed drying, sieving, blending with HPMC, lubrication by addition of magnesium stearate, and tableting (Fig. 2B).

2.2. Analytical methods

2.2.1. Determination of NDMA

The content of NDMA in drug substance and different drug products was determined by GC–MS/MS and LC-MS/MS. Good quantitative agreement between these orthogonal methods had previously been demonstrated (Fritzsche et al., 2021). In general, both methods have been used to measure the samples throughout the manuscript, except results presented in section 3.8.1, which were all obtained by GC–MS/MS and results presented in section 3.7, which were all obtained by LC-MS/MS. Results shown in section 3.1 were obtained by LC-MS/MS for starting material and intermediates and by GC–MS/MS for finished API.

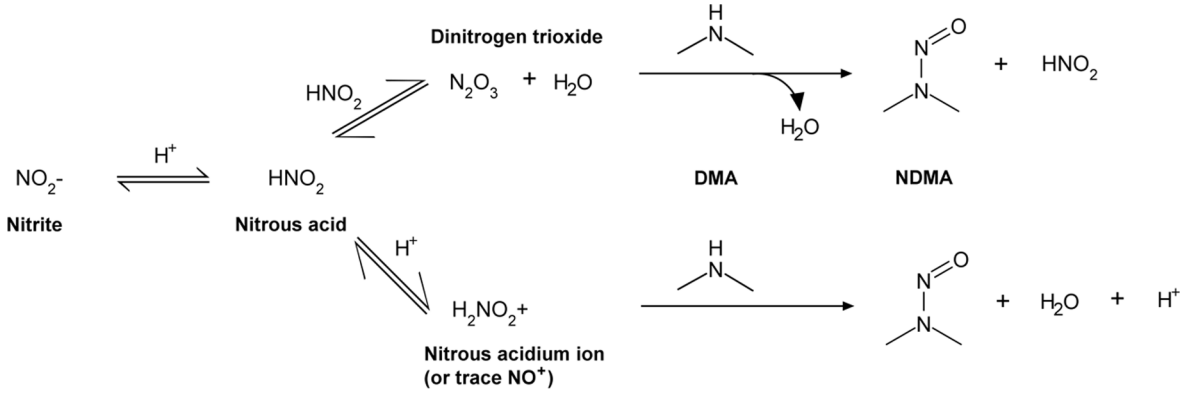

Fig. 1. Formation of NDMA from DMA and nitrite. The actual nitrosating agent can either be the nitrous acidium ion, the nitrosonium ion or dinitrogen trioxide, depending on the actual reaction conditions. Adapted from (Fritzsche et al., 2021).

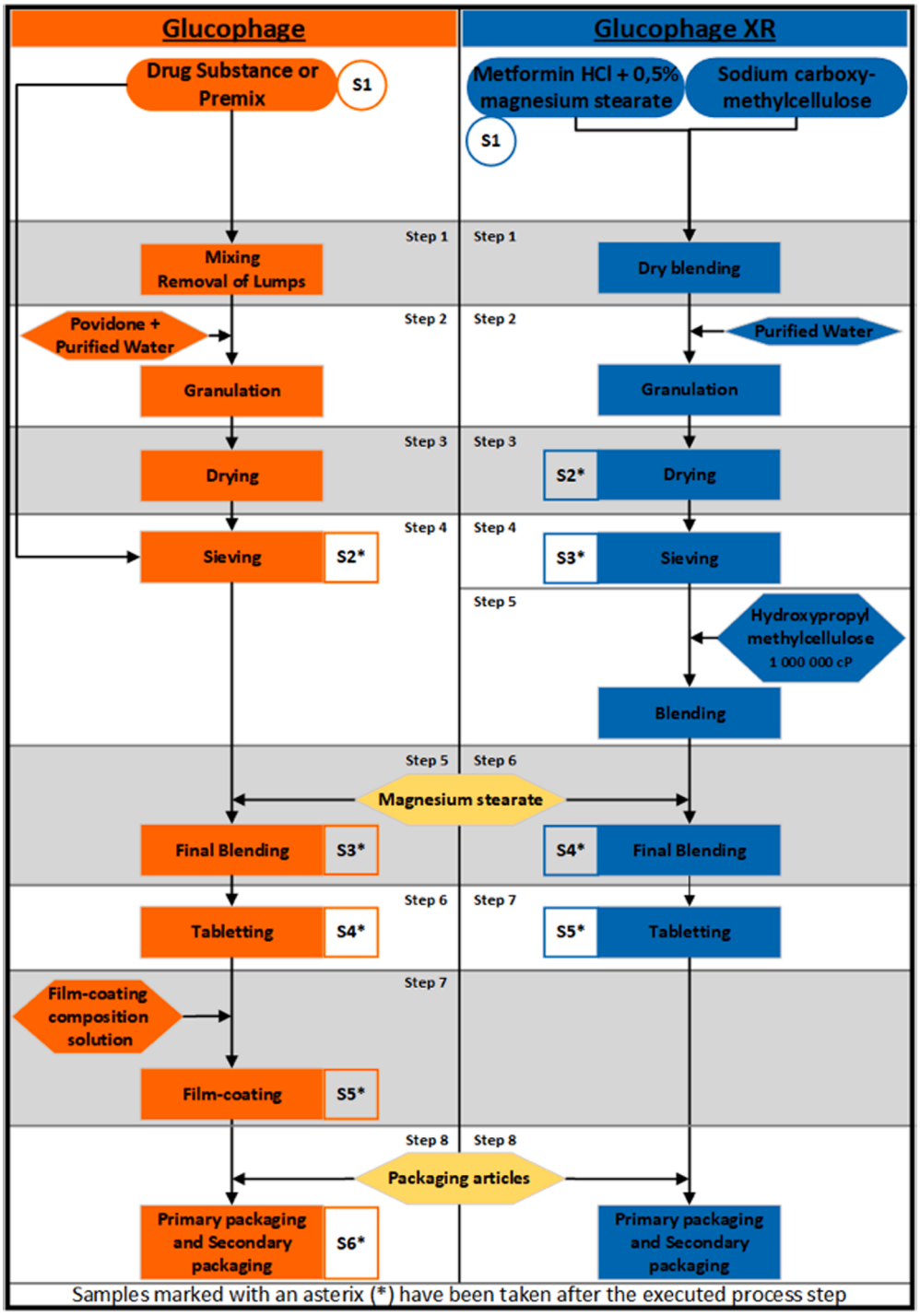

Fig. 2. Flow diagram of (A) Glucophage® and (B) Glucophage® XR manufacturing processes.

2.2.1.1. Sample homogenization. All samples were ground using an IKA Tube Mill Control (10000 rpm, 15 s interval, 1:17 min total time) with disposable milling chambers (IKA, Staufen, Germany) until a fine powder was obtained. Milled samples were used immediately to avoid loss of NDMA through evaporation.

2.2.1.2. LC-MS/MS method for analysis of NDMA in Metformin API, API intermediates (in-process samples) and Glucophage®. Approximately 100 mg powder were weighed into a 5 mL Eppendorf tube. 1.0 mL water (LiChrosolv, Merck Millipore, Burlington, USA) and 50 µL of a 250 ng/mL NDMA-d6 (CDN Isotopes, Pointe-Claire, Canada) internal standard in water were added. The mixture was shaken well for 5 min at maximum speed (3200 rpm) on a mechanical vortex shaker (Vortex-Genie 2, Scientific Industries Inc., New York, USA). Then 1.0 mL of dichloromethane (for analysis Emsure, Merck Millipore, Burlington, USA) was added, and the tube was again vortexed for 5 min at maximum speed (3200 rpm). The suspension was centrifuged for at least 5 min at 4000 rpm, and 200 µL of the lower DCM phase was transferred into another Eppendorf tube. To this, 50 µL water was added and the DCM was removed through gentle shaking at 25 °C on a thermo shaker (Universal Labortechnik, Leipzig, Germany) at 1500 rpm with open lid of the cup for about 90 min until the DCM was fully evaporated. Then 150 µL water was added, the mixture was shaken briefly and transferred into a suitable vial for analysis. Quantitation was performed using a six-

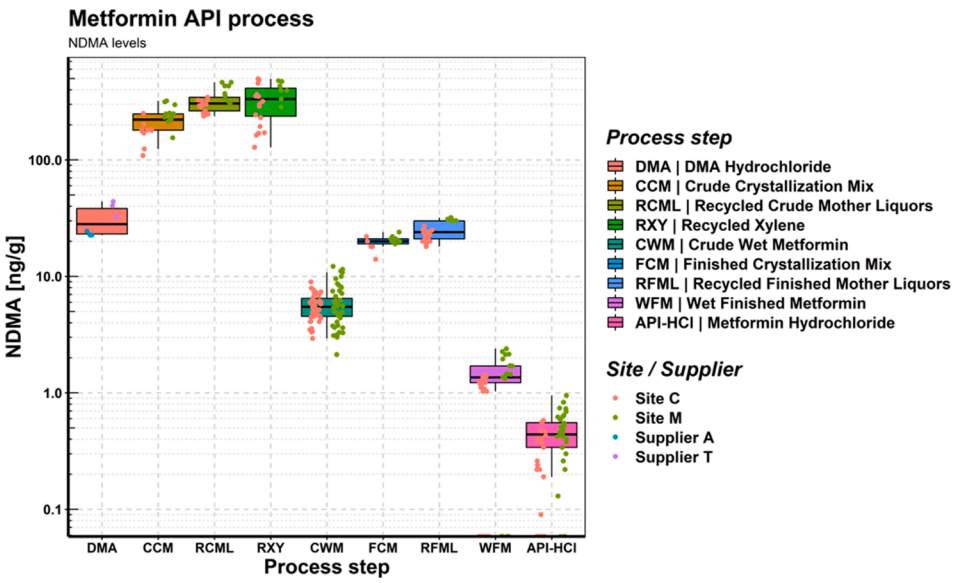

Fig. 3. NDMA content (ng/g sample) at different steps of the Metformin manufacturing process. NDMA was analyzed in 3 batches each of DMA hydrochloride from different suppliers, as well as in 10 samples CCM from site C and 13 samples from site M, 16 samples RCML from site C and 9 samples from site M, 16 samples RXY from site C and 7 samples from site M, 50 samples CWM from site C and 41 samples from site M, 5 samples FCM from site C and 8 samples from site M, 25 samples WFM from site C and 21 samples from site M and 30 batches each of finished Metformin hydrochloride from sites C and M. API samples were from more recent batches than the in-process samples.

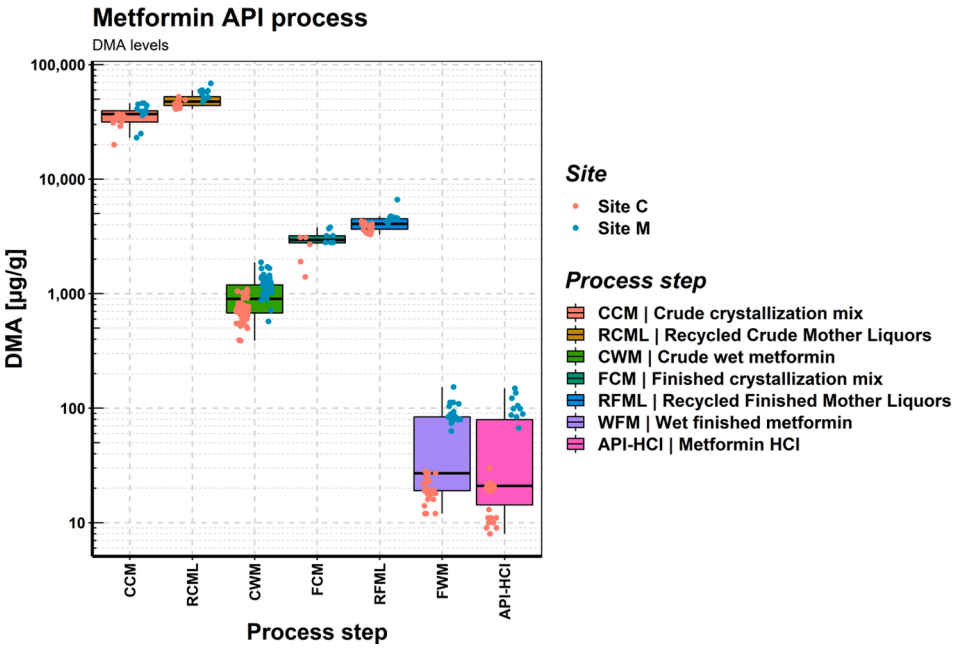

Fig. 4. DMA content (µg/g sample) at different steps of the Metformin manufacturing process. DMA was analyzed in 10 samples CCM from site C and 13 samples from site M, 16 samples RCML from site C and 10 samples from site M, 46 samples CWM from site C and 41 samples from site M, 5 samples FCM from site C and 7 samples from site M, 16 samples RFML from site C and 10 from site M, 25 samples FWM from site C and 21 samples from site M, as well as 30 batches each of finished Metformin hydrochloride from sites C and M. Note that samples from site M were taken before the implementation of measures to reduce DMA, as described in 3.4. Furthermore, API samples from site M were taken after an additional processing step, i.e., blending with 0.5% magnesium stearate, which is unlikely to affect the DMA level.

Table 1
Composition of Glucophage® and Glucophage® XR tablets (modified from Fritzsche et al. (Fritzsche et al., 2021)).

Component	% (m/m) of Glucophage®	% (m/m) of Glucophage® XR	% (m/m) of Glucophage® XR OF	Nitrite found [ppm]
Metformin HCl	94.5	69.0	48.5	0.01 – 0.05 (n = 43)[1]
Povidone K30	3.8	–	–	0.1 – 2.5 (n = 24)[2]
Magnesium stearate	0.9	0.5	0.6	0 – 1.7 (n = 24)[1]
Carmellose sodium	–	3.4	4.9	<0.03 (n = 2)[1]
HPMC 100 k	–	27.1	35.0	0.01 – 3.7 (n = 19)[2]
HPMC E5	–	–	1.0	0 – 0.54 (n = 18) [1]
Microcrystalline cellulose	–	–	10.0	0 – 0.24 (n = 14) [1]
Coating	0.8	–	–	<0.03 (n = 5)[1]

[1] *data not shown*
[2] Fig. 9

point calibration covering the range of 0.5 ng/mL (corresponding to 5 ng/g in sample) to 50 ng/mL (corresponding to 500 ng/g in sample) NDMA (Merck Millipore, Burlington, USA) on an Acquity H-Class UPLC (Waters, Milford, USA) coupled to an API 6500 + tandem mass spectrometer (ABSciex, Framingham, USA) in APCI positive mode using an ACE Excel 1.7 C18-AR 100 × 2.1 mm column (Cat.No. EXL-179-1002U;

Advanced Chromatography Technologies Ltd, Aberdeen, UK). Quantifier transition of NDMA was $m/z = 75.1$ to $m/z = 43.1$ (dwell time: 200 s, CE: 21 eV), qualifier transition was $m/z = 75.1$ to $m/z = 58.0$ (dwell time: 200 s, CE: 17 eV).

2.2.1.3. Alternative LC-MS/MS method for analysis of NDMA in Metformin API and DP. Metformin API and Glucophage®: Approximately 500 mg powder were weighed into a 5 mL glass volumetric flask. 100 µL of a 500 ng/mL NDMA-d6 (Clearsynth, Mumbai, India) internal standard in water were added and the sample was vortexed for 30 s. Then the flask was filled to volume with water (ULC/MS; Biosolve, Dieuze, France), and the solution was stirred for 10 min. An aliquot of the suspension was taken and filtered using a 0.2 µm PVDF syringe filter (Pall, New York, USA) prior to analysis. Quantitation was performed using a seven-point calibration covering the range of 1 ng/mL (corresponding to 10 ng/g in sample) to 12 ng/mL (corresponding to 120 ng/g in sample) NDMA on an Acquity UPLC (Waters, Milford, USA) coupled to a Xevo TQ-S tandem mass spectrometer (Waters, Milford, USA). Quantifier transition of NDMA was $m/z = 75.0$ to $m/z = 58.0$ (CE: 20 eV). Further method details were published previously (Fritzsche et al., 2021).

Glucophage® XR: Approximately 100 mg powder were weighed into a 5 mL glass tube. 20 µL of a 500 ng/mL NDMA-d6 (Clearsynth, Mumbai, India) internal standard in water were added, and the sample was vortexed for 30 s. Then, 1.0 mL DCM (GC grade, Merck Millipore, Burlington, USA) was added to the tube. The tube was shaken laterally for 10 min. 2.0 mL water (ULC/MS; Biosolve, Dieuze, France) was added and the mixture was vortexed for 2 s and centrifuged for 10 min at 5000 g at 5 °C. The DCM layer was transferred into a clean 5 mL tube. 5 µL formic acid and 250 µL water were added. The DCM was evaporated under a stream of nitrogen, ensuring the water was always present to trap the NDMA. The solution was then reconstituted in 0.5 mL water/methanol (95/5) v/v (ULC/MS; Biosolve, Dieuze, France) and an aliquot of the solution was filtered using a 0.2 µm PVDF syringe filter (Pall, New York, USA) prior analysis. Quantitation was performed using a seven-point calibration covering the range of 2 ng/mL (corresponding to 10 ng/g in sample) to 24 ng/mL (corresponding to 120 ng/g in sample) NDMA on an Acquity UPLC (Waters, Milford, USA) coupled to a Xevo TQ-S tandem mass spectrometer (Waters, Milford, USA). Quantifier transition of NDMA was $m/z = 75.0$ to $m/z = 58.0$ (CE: 20 eV). Further method details were published previously (Fritzsche et al., 2021).

2.2.1.4. GC–MS/MS method for analysis of NDMA in Metformin API, DP, and DP intermediates. Metformin drug substance, Glucophage® and drug product intermediates without HPMC: Approximately 250 mg powder were weighed into a 15 mL centrifuge tube. 10 mL of aqueous solution (LiChrosolv; Merck Millipore, Burlington, USA), containing NDEA-d4 (TRC, Toronto, Canada) internal standard at 2.5 ng/mL in water was added. This was briefly vortexed and shaken manually for 5 min. Then 2.0 mL of DCM (SupraSolv; Merck Millipore, Burlington, USA) was added. The suspension was vortexed briefly and shaken well for 5 min. If necessary, the suspension was centrifuged, and the lower DCM phase was taken for analysis.

Glucophage® XR and drug product intermediates containing HPMC: Approximately 500 mg powder were weighed into a 15 mL centrifuge tube. 4.0 mL of DCM (SupraSolv; Merck Millipore, Burlington, USA), containing NDEA-d4 (TRC, Toronto, Canada) internal standard at 12.5 ng/mL was added. The mixture was vortexed for 1 min. 8.0 mL water (LiChrosolv; Merck Millipore, Burlington, USA) was added and vortexed briefly prior to centrifuging for 3 min at 4000 rpm. The DCM layer was then transferred into a suitable vial for analysis.

Quantitation was performed using a three-point calibration with solutions that were prepared the same way as the corresponding sample solutions, containing NDMA (EPA 8270 mix, Merck Millipore, Burlington, USA) and covering the range of 1.875 ng/mL (corresponding to 15 ng/g in sample) to 15.000 ng/mL (corresponding to 120 ng/g in sample) of NDMA. The analyses were carried out on a GC7890 gas chromatograph (Agilent, Santa Clara, USA) combined with a 7000B tandem mass spectrometer (Agilent, Santa Clara, USA). Column was a DB-624 (30 m, 0.25 ID, 1.4 µm film), injection 2 µL pulsed splitless. Quantifier transition of NDMA was $m/z = 74$ to $m/z = 44$ (dwell time: 200 s, CE: 5 eV), qualifier transition was $m/z = 74$ to $m/z = 42$ (dwell time: 200 s, CE: 22 eV). A comparison of methods 2.1.2.2, 2.1.2.3 and 2.1.2.4 as well as further method details and validation results were published previously (Fritzsche et al., 2021).

2.2.1.5. Determination of DMA in Metformin API (QC test for batch release). 500 mg Metformin API was weighed into a 25 mL volumetric flask, mixed with 10 mL ultrapure water (LiChrosolv; Merck Millipore, Burlington, USA) and sonicated until complete dissolution. 1.0 mL 0.1 M NaOH (Merck Millipore, Burlington, USA) and 600 mg 4-Nitrobenzoylchloride derivatization reagent (LiChropur, Merck Millipore, Burlington, USA) were added, stirred with a magnetic stirrer for 5 min and filled to volume with 40% acetonitrile (Gradient grade, Merck Millipore, Burlington, USA). The solution was passed through Minisart RC25 syringe filters (Sartorius, Goettingen, Germany). 2 mL filtrate was transferred into 10 mL volumetric flasks and filled to volume with 40% acetonitrile. Aliquots were transferred to autosampler vials. 10 µL sample was analyzed on an UltiMate 3000 HPLC-UV system (Thermo Fisher Scientific, Waltham, USA) equipped with an Uptisphere C18 column (150 × 4.6 mm, 3 µm, Interchim, Montluçon, France). Isocratic elution was performed with 40% acetonitrile at a constant flow rate of 1.0 mL/min. Chromatograms were recorded at 280 nm. Elution of derivatized DMA occurred after ca. 4 min. DMA content in samples was calculated by comparison to 18.12 µg/mL DMA HCl (>99%, Merck Millipore, Burlington, USA) standard solution (corresponding to 0.05% = 500 µg/g DMA base in sample), derivatized in the same way as the samples. Single-point calibration was used as an excellent linearity of the response ($R^2 > 0.999$) in the range of 0.3624 µg/mL to 36.24 µg/mL DMA HCl (corresponding to 10 µg/g to 1000 µg/g DMA base in sample) had been demonstrated during method validation (data not shown).

2.2.1.6. Determination of DMA in Metformin API and PD in-process samples by ion chromatography with conductivity detection. All chemical, solvents and consumables were obtained from Merck Millipore (Burlington, USA), if not indicated otherwise. Ultra-pure water was prepared on an IQ7000 system (Merck Millipore, Burlington, USA).

Solid samples were finely ground using a laboratory mill (see 2.1.2.1 Sample homogenization). 250 mg of powder or of solution (in case of mother liquors) were exactly weighed into a 25 mL volumetric flask and dissolved in about 20 mL ultra-pure water by shaking. Some excipients like magnesium stearate did not dissolve under these conditions and left a visible undissolved residue. The flask was filled to the mark with water. All samples were filtered over a 0.45 µm PFTE syringe filter. A 0.5 mg/L DMA standard in ultra-pure water was prepared by dissolving and diluting DMA-hydrochloride, appropriately.

Samples and calibrants were analyzed on an ICS 6000 system (Thermo Fisher Scientific, Waltham, USA). 50 µL were injected on an IonPac CS10 column (4 × 250 mm) equipped with an IonPac CG10 guard column (4 × 50 mm, both Thermo Fisher Scientific, Waltham, USA). DMA was eluted isocratically at 1.0 mL/min at room temperature with 40 mM methanesulfonic acid in ultra-pure water under nitrogen gas. DMA eluted around 17 min and was detected by suppressed conductivity (120 mA). Excellent linearity ($R^2 > 0.999$) between 10 µg/g and 1000 µg/g was achieved with a seven-point calibration (data not shown). Therefore single-point calibration was used for routine testing. All samples and standards were injected three times, and the mean values used for calculation of DMA content. Samples exceeding 1000 µg/g were diluted accordingly and re-analyzed.

2.2.2. Determination of nitrite

2.2.2.1. Determination of nitrite in povidone and HPMC 100 K using ion chromatography. Ion chromatography (IC) with conductivity detection has been described for the determination of both nitrite and nitrate in various matrices in literature (Moorcroft, 2001).

All chemical, solvents and consumables were obtained from Merck Millipore (Burlington, USA), if not indicated otherwise. Ultra-pure water was prepared on an IQ7000 system (Merck Millipore, Burlington, USA).

2500 mg of HPMC 100 K samples were exactly weighed into a 50 mL volumetric flask. About 40 mL of extraction solvent (acetonitrile + methanol, 3 + 1, v/v) were added and the sample was shaken for five minutes. Afterwards, the flask was brought to volume with extraction solvent and filtered through a 0.45 µm PTFE membrane filter. To remove interfering chloride matrix, present in HPMC 100 K the filtrate was filtered a second time over a silver loaded cartridge filter (Dionex OnGuard II Ag cartridges, 1 mL, Thermo Fisher Scientific, Waltham, USA). These Ag-cartridges were rinsed with 15 mL ultra-pure water followed by 3 mL of air at least 24 h prior to use. Immediately before sample filtration, they were rinsed again with 15 mL of ultra-pure water followed by 3 mL of air. Afterwards, 2 mL of sample filtrate were slowly passed through the cartridge, and the initial filtrate was discarded. Then, 25–30 mL of sample filtrate were passed through the cartridge drop by drop. Finally, 10 mL of the obtained second filtrate were pipetted in a 20 mL volumetric flask an brought to volume with 1.3 mM $NaHCO_3$ and 1.4 mM Na_2CO_3 in ultra-pure water. Standard addition was used for quantitation. To that end, 10 mL of the filtrate after Ag-filtration were pipetted in a 20 mL volumetric flask and 500 µL of a 10 mg/L nitrite and nitrate in ultra-pure water, corresponding to an addition of 10 µg/g were added. Afterwards, the flask was brought to volume with 1.3 mM $NaHCO_3$ and 1.4 mM Na_2CO_3 in ultra-pure water. A blank solution was prepared by mixing equal amounts of extraction solvent and 1.3 mM $NaHCO_3$ / 1.4 mM Na_2CO_3 solution.

Povidone samples were as well analyzed using standard addition. To that end, 500 mg of sample were exactly weighed into a 10 mL volumetric flask, fully dissolved in sample solvent (1.3 mM $NaHCO_3$ and 1.4 mM Na_2CO_3 in ultra-pure water) and spiked with 100 µL of an aqueous 10 mg/mL nitrite and nitrate standard solution, corresponding to an addition of 2 mg/kg. Afterwards, the flasks were brought to volume with sample solvent. Non-spiked samples were prepared analogously without the addition of nitrite/nitrate standard. Sample solvent was used as a blank sample.

Both HPMC 100 K as well as povidone samples were analyzed using the same chromatographic conditions.

Sample solutions, spiked sample solutions and blanks were injected with a large volume sample loop (778 µL) into the IC system (ICS 3000, Thermo Fisher Scientific, Waltham, USA) equipped with an IonPac AG23 guard column (4 × 50 mm) and an IonPac AS23 analytical column (4 × 250 mm, both columns Thermo Fisher Scientific, Waltham, USA). The column compartment was kept at 30 °C and the sample was eluted isocratically with 1.3 mM $NaHCO_3$ and 1.4 mM Na_2CO_3 in ultra-pure water at 1.0 mL min (runtime 40 min). Nitrite and nitrate were detected with suppressed conductivity (50 mA). Recorded data was evaluated using blank subtraction.

2.2.2.2. Determination of nitrite in Metformin DP after Griess reaction. Nitrite can form an azo dye with sulfanilic acid and *N*-(1-naphtyl)ethylenediamine under acidic conditions as described in literature (Griess, 1879). This reaction is long known as "Griess reaction", named after its inventor Peter Griess. While the original publication uses naphthylamine, *N*-(1-naphtyl)ethylenediamine is the more common reaction partner today (Moorcroft, 2001). Typically, this method is used to detect nitrite with UV-spectroscopy. We have added a chromatographic step to separate the azo dye from interfering matrix. As the reaction is specific for nitrite, no nitrate values can be measured with this approach and nitrate does not interfere. All chemical, solvents and consumables were obtained from Merck Millipore (Burlington, USA), if not indicated otherwise. Ultra-pure water was prepared on an IQ7000 system (Merck Millipore, Burlington, USA).

1000 mg of ground sample was accurately weighed into a 15 mL polypropylene tube. After addition of 10.0 mL of extraction solvent, the samples were shaken on a vortex shaker (Multitube vortex mixer digital, Thermo Fisher Scientific, Waltham, USA) for five minutes.

Ultra-pure water was used as extraction solvent for Glucophage®, methanol for Glucophage® XR. Resulting suspensions were centrifuged for five minutes at 3000 rpm after shaking. 750 µL of supernatant was transferred into an autosampler vial and 100 µL of ultra-pure water, 50 µL of 37% aqueous HCl, 50 µL of a 10 mg/mL solution of sulfanilic acid in ultra-pure water (reagent A) and 50 µL of a 10 mg/mL solution of N-(1-naphtyl)ethylendiamine-hydrochloride in ultra-pure water (reagent B) were added. The solutions of sulfanilic acid and N-(1-naphtyl)ethylendiamine-hydrochloride were prepared in amber glassware. Four different spiking levels per sample were prepared for quantitation via standard addition. To that end, 750 µL of the sample supernatant were spiked individually with 100 µL of an aqueous nitrite solution with 50, 100, 500 and 1000 ng/mL (corresponding to 66.6–1333 ng/g nitrite in the solid sample) in autosampler vials. Afterwards, 50 µL each of 37% aqueous HCl, reagent A and reagent B were added. A reagent blank sample was prepared by addition of 50 µL each of 37% HCl, reagent A and reagent B to 850 µL of respective extraction solvent in an autosampler vial. Samples (as well as blanks and spiked samples) were analyzed on a Hitachi LaChrom Elite HPLC-UV system (Hitachi Ltd, Tokyo, Japan). 99 µL of sample solution was injected onto a Purosphere STAR RP18e column (150 × 3 mm, 3 µm) operated at 40 °C. A binary gradient at a constant flow rate of 1.0 mL/min with water and 0.1% formic acid (v/v, eluent A) and acetonitrile with 0.1% formic acid (v/v, eluent B) was applied. 10% B were kept for 0.5 min and increased to 80% B at 4.0 min which was kept constant for 0.5 min. After returning to the initial 10% B in 0.1 min, the system was equilibrated for 3.4 min prior to the next injection. The azo dye eluted at 4.7 min and was used for quantification by UV detection at 485 nm.

Chromatograms were integrated, and the nitrite levels calculated after blank subtraction using standard addition with linear fit and weighting with reciprocal amount.

2.2.3. Loss on drying

Analysis was performed as described in the Ph.Eur. chapter 2.2.32 "Loss on drying" (EDQM, 2022a), using an MA150C digital moisture scale (Sartorius, Goettingen, Germany). 10 g of sample were heated to 85 °C for 10 min. The loss on drying is the difference in the mass of the sample before and after drying, expressed as a percentage.

2.2.4. Determination of peroxide in povidone

UV/Vis spectra were acquired using a Specord 200 plus UV/Vis spectrometer (Analytik Jena, Jena, Germany). Absorption was measured at 405 nm. Water was used as reference for all measurements.

For quantification of peroxide, the compendial method described in Ph.Eur. monograph "Povidone" (EDQM, 2022b), was adopted and modified.

2 g povidone were diluted with 30 mL water in a 50 mL flask. 4 mL of titanium trichloride-sulfuric acid reagent R (VWR Chemicals) (Ph. Eur. 1091202; EDQM, Strasbourg, France) was added and the flask was filled with water. As blank, 2 g povidone were diluted with 30 mL water in a 50 mL flask. 4 mL of 13% v/v sulfuric acid (Merck Millipore, Burlington, USA) was added, and the flask was filled with water.

The absorptions of the sample and blank values are normalized to the intended sample weight. A calibration curve was built using hydrogen peroxide (Merck Millipore, Burlington, USA). Six standards in the concentration range of 0 ppm to 20 ppm were used for calibration reaching a linear correlation of $R^2 > 0.999$.

The concentration was calculated according to the following equa-

tion:

$$concentration = \frac{[(A_{sample} - A_{blank}) - b]}{\varepsilon \bullet d} \bullet 25$$

A_{sample} absorption sample (normalized).
A_{blank} absorption blank (normalized).
25 Dilution factor of solution.
b intercept of y-axis.
ε slope – extinction coefficient.
d Optical pathlength in the cuvette.

2.3. Statistical analyses

2.3.1. Methodology for assignment of purge factors using Mirabilis

The Mirabilis software tool (version 3.2.0.20, Lhasa Limited) was used to assess purge factors for the carcinogenic impurity N-dimethylnitrosamine (NDMA) based on physicochemical parameters and process conditions used for Metformin manufacture in line with the provision within ICH M7 guidance for the control of impurities using key risk-based principles.

This technique used standardized, consistent, and reproducible calculations to define the degree of "purge" predicted for NDMA in relation to the manufacture of Metformin Pharmaceuticals. The established scoring system is used to derive an understanding of the extent of removal of NDMA through evaluation of the process parameters and the reactivity, solubility, and volatility of NDMA.

Expert elicitation for reactions within Mirabilis, were previously established and a "low reactivity" purge factor of 1 is obtained from the reactivity matrix for NDMA under the reaction conditions used for Metformin manufacture. Expert elicitation for solubility within Mirabilis, relies on understanding of the difference in solubility between the impurity and the solid product at each stage. Since the solubility of NDMA is aqueous systems is well established to be around over 100 mg/mL, it is concluded to be freely soluble, with a purge factor of 10 for each stage of the Metformin production process. Finally, expert elicitation for volatility within Mirabilis was considered and as the boiling point of NDMA is $>$ 20 °C above that of the process solvent (water) to be removed in drying, a purge factor of 1 is established as appropriately conservative.

2.4. Data analysis and visualization

Statistical data analysis and visualization were done with the R language for statistical computing version 4.1.1 (R_Core_Team, 2021) using the packages ggplot2 version 3.3.5 (Wickham, 2016), ggpubr version 0.4.0.999 (Kassambara, 2020), patchwork version 1.1.1 (Pedersen, 2020) and ggpmisc version 0.4.3 (Aphalo, 2021).

3. Results and discussion

3.1. Depletion of NDMA introduced during API manufacturing by double crystallization

Synthesis of Metformin is done by reacting 2-cyanoguanidine with DMA hydrochloride. To transfer the Metformin into an aqueous environment for crystallization, aqueous crude mother liquors from previous crystallization are added to the reaction mixture. This yields the crude crystallization mix (CCM). The CCM is centrifuged to yield the crude wet Metformin (CWM). CWM is then dissolved in finished mother liquors recovered from previous recrystallizations, yielding the finished crystallization mix (FCM), from which Metformin is crystallized. The product is centrifuged to yield the wet finished Metformin (WFM). Finally, the product is dried and transferred to a blender for homogenization, resulting in the final Metformin hydrochloride API.

Samples from these steps were collected and analyzed for NDMA by LC-MS/MS. The highest measured content was ca. 200–300 ng/g in the crude crystallization mix (CCM). Results from samples from downstream steps, i.e., 2x crystallization and 1x drying, showed that NDMA is continuously depleted to a final safe level of $<$ 1 ng/g in a consistent manner (Fig. 3). The depletion of NDMA can be ascribed to the high solubility of NDMA in aqueous media as well as to its volatility during the drying process. The only stages at which the NDMA content increased were the crystallization mixes, for which recycled mother liquors that contain NDMA from previous cycles are introduced. The contents that were reached correspond to those of the respective mother liquors, indicating that the NDMA in the system may not be formed from DMA during the synthesis process but rather introduced by the starting material DMA hydrochloride and gradually enriched in the recycled solvents. Solvent recycling is required to extract residual Metformin, which remains at ca. 300 g/L after crystallization.

The variability of NDMA content at respective process steps was low both between batches from each site and between the alternative manufacturing sites C and M, indicating high process robustness with regards to NDMA depletion. Based on these findings, if$>$1 ng/g NDMA is found in a Metformin drug product, it must have developed during the drug product manufacturing, packaging, or storage. As NDMA can be formed from DMA and nitrosating agents as shown in Fig. 1, the API's DMA content was likely to be an important factor and was determined in analogy to NDMA at the respective steps of the API manufacturing process (Fig. 4). Like for NDMA, also afor DMA a continuous depletion along the process was observed, starting at 30000–40000 μg/g in the crude crystallization mix, down to a level of 10–100 μg/g in the final API. As for NDMA, the only content increase occurs at the FCM stage and can be ascribed to the same reason, the introduction of mother liquors from previous batches. While the variability of DMA content between batches from the same site was low at all stages of the process, DMA levels in the FWM and final API were about one order of magnitude higher in batches from site M compared to manufacturing site C. Note that the data from site M were recorded before implementation of the improvements presented in section 3.4.

3.1.1. Comparison of measured NDMA & DMA purge factors and respective in-silico predictions

Mirabilis (Burns et al., 2019; Burns et al., 2020) is a software tool developed by the Mirabilis Consortium that predicts purge factors for potentially mutagenic impurities based on physicochemical parameters and process conditions (Teasdale et al., 2013). A purge factor is defined as the level of an impurity at an upstream process stage divided by the level of that impurity at a downstream process stage.

We used Mirabilis to calculate purge factors for the different unit

Table 2
Comparison of experimentally determined purge factors and purge factors calculated via the software tool Mirabilis. The contents shown are average values calculated from the data shown in Fig. 3 and Fig. 4, and the purge factors are calculated based on these average values.

	NDMA [ng/g]		DMA [μg/g]	
	Site M	Site C	Site M	Site C
CCM	247	176	38,385	31,900
Purge1 experimental	40	32	32	45
Purge1 Mirabilis	10		10	
CWM	6.1	5.4	1201	708
Purge2 experimental	0.3	0.3	0.4	0.3
Purge2 Mirabilis	n/a		n/a	
FCM	21	18	3186	2240
Purge3 experimental	19	27	35	115
Purge3 Mirabilis	10		10	
WFM	1.1	0.7	91	20
Purge4 experimental	2.4	3.6	0.9	1.1
Purge4 Mirabilis	1		1	
API	0.5	0.2	104	17
Total purge experimental	***545***	***935***	***370***	***1872***
Total purge Mirabilis	***100***		***100***	

operations of the Metformin manufacturing process, as summarized in Table 2. With respect to NDMA, Mirabilis allowed a purge factor of 10 to be assigned for each of the two crystallizations, and a purge factor of 1 for the drying step that yields the finished API, resulting in a total purge factor of 100. This compares with the experimentally determined total purge of ca. 545 for site C and 935 for site M. With respect to DMA, the same factor of 10 was assigned to the two crystallization steps, and again no purge was assigned to drying step, due to the low volatility of DMA hydrochloride, leading to a predicted total purge factor of 100. The experimentally determined total purge factor was 370 for site M and 1872 for site C. Mirabilis gave conservative estimations of the individual purge factors, underestimating the experimentally determined values 1.8-fold to 10-fold. Even the total purge was underestimated by Mirabilis, although the interim increase of NDMA and DMA caused by the addition of recycled mother liquors was not included in the calculation of the predicted purge factors. It should be noted that the measured NDMA contents of WFM and API are below LOQ and hence only estimations of the actual values, likewise the experimental purge factors calculated based on those contents. We conclude that the Mirabilis software is highly useful to derive conservative estimations of processes' purge capacities.

3.2. NDMA formation in the drug product manufacturing process

As demonstrated in section 3.1, the content of NDMA in the API is negligible. In addition, the analysis of the excipients used for the manufacture of Glucophage® and Glucophage® XR could exclude those as the source of NDMA as well (data not shown). However, the presence of NDMA had been confirmed for drug product batches. For these reasons, the formation of NDMA must occur during drug product manufacturing, which is assessed in this section, and/or during storage, which is summarized in section 3.7. Technical batches of both drug products at commercial scale were made to assess the impact of the manufacturing processes and to identify those steps that promote NDMA formation (Fig. 5, Fig. 6).

With respect to Glucophage®, NDMA content increased during wet granulation and coating. The wet granulation step introduces heat and PVP K30 as a significant source of nitrite (refer to Table 1). The coating process introduces heat and moisture. Blending the granules reduced the content of NDMA slightly ad interim, possibly due to evaporation of the NDMA. Using PVP K30 from supplier B resulted in the same pattern of NDMA formation compared with supplier A, but the levels reached at each manufacturing step were distinctly lower (see also section 3.3.1). Furthermore, it could be demonstrated that the primary packaging into PVC-Alu blisters does not contribute to NDMA in the finished product (Fig. 6). It is to be noted that there is a risk for NDMA formation through primary packaging if the tablet comes into contact with the ink which contains nitrocellulose (EMA/CHMP, 2020), see also section 3.7.

With respect to Glucophage® XR, NDMA is kept at low level until introduction of HPMC 100 k in the final blend. Like PVP in Glucophage® tablets, HPMC had been identified as the main contributor of nitrite to Glucophage® XR tablets. Tableting of the granules led to a further increase of NDMA, possibly due to the generation of heat and mechanical stress through the compression.

3.3. Influencing factors of NDMA formation in drug product manufacturing

We observed high variability of NDMA results in Glucophage® and Glucophage® XR tablet batches and found the following factors to have the clearest impact on the amount of NDMA found.

3.3.1. Glucophage®

For Glucophage® tablets, there was a strong correlation of API DMA content and NDMA found in the finished product if PVP from supplier A was used. When supplier B was used, NDMA was consistently low < 15 ng/g, regardless of the DMA content in the API. Respective data is summarized in Fig. 7.

3.3.2. Glucophage® XR

For Glucophage® XR, we noted that the formulation variant with lower loss on drying after granulation, called the "old formulation" (OF) had consistently low NDMA across all tested drug product batches, regardless of the API's DMA content. The formulation variant with higher loss on drying after granulation, the so-called "reduced mass" (RM) formulation, had high NDMA content particularly if HPMC 100 k excipient from supplier A was used. With HPMC from supplier D, NDMA was distinctly lower. It is unclear if the different behavior between OF and RM, which continues along the products' shelf-life (3.7.3) is due to differences in the water content, the presence of microcrystalline cellulose as an additional excipient in the old tablet formulation or yet another non-obvious difference. Water had previously been named by Nasr *et al.* as potential factor supporting the formation of NDMA in Metformin drugs (Nasr et al., 2021). Except for Fig. 8, all Glucophage® XR data shown are for the reduced mass formulation.

3.3.3. Influence of nitrite levels in the excipients PVP and HPMC

PVP is used as an excipient in Glucophage®, whereas HPMC is used in Glucophage® XR (Table 1). This paragraph summarizes effects of NDMA found in both excipients from different suppliers. PVP was

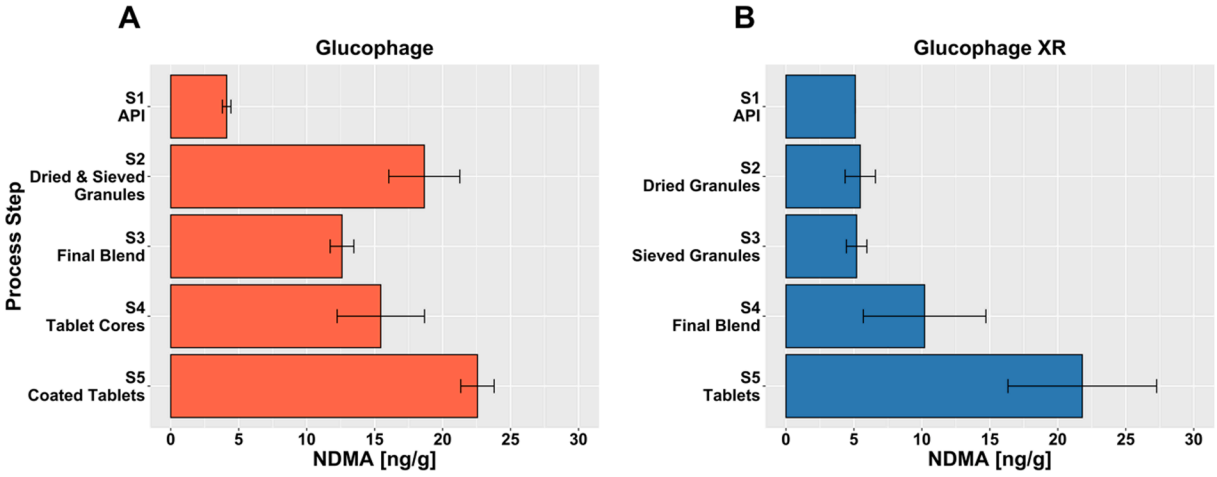

Fig. 5. Formation of NDMA in the production process of Glucophage® (immediate release formulation) & Glucophage® XR (extended-release formulation). Each graph shows the average from the production of three commercial-scale batches made before implementation of any improvements described below.

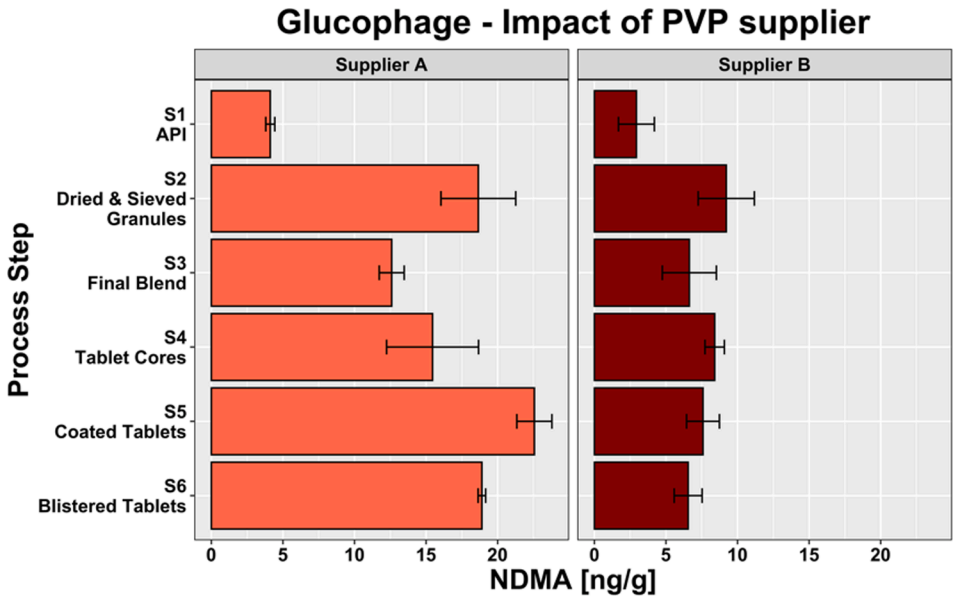

Fig. 6. Comparison of NDMA formation using PVP from different suppliers. PVP from supplier A was identified to contain higher content of nitrite. The same pattern of NDMA formation is observed, but the levels reached are lower with supplier B compared with supplier A. Furthermore, it is shown that the packaging process does not increase the NDMA content.

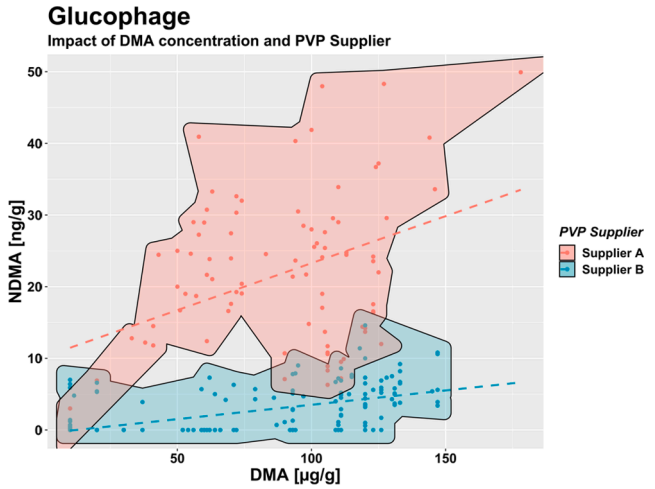

Fig. 7. More NDMA and higher variability of NDMA in Glucophage® batches produced with PVP from supplier A, compared with supplier B.

purchased from suppliers A and B while HPMC was obtained from suppliers A, C and S. The increased NDMA formation observed with HPMC and PVP excipient from supplier A could be shown to be linked with a higher nitrite content. For both excipients, the nitrite content is higher and more variable in batches from supplier A compared with batches from the alternative suppliers C and S (HPMC) and B (PVP) (Fig. 9). Therefore, the use of HPMC and PVP excipients from supplier A was immediately discontinued. The nitrite content is the most important factor for the formation of NDMA through nitrosation of DMA in Metformin drug products, as previously described (Fritzsche et al., 2021). Therefore, limiting nitrite from excipients must be a key element of any NDMA control strategy. PVP had previously been highlighted as potential contributor to the formation of NDMA in Metformin drugs by Jireš *et al.* (Jireš et al., 2021), but the authors attributed the effect to the presence of peroxide in that excipient as highlighted by Wu *et al.* (Wu et al., 2011), because they found higher NDMA content in technical batches made with PVP that they assumed to contain more peroxide (32 µg/g vs 165 µg/g). However, the lower value was taken from the manufacturer's COA, whereas the higher value was determined in-house. Furthermore, the authors did not check for differences in nitrite content between the low-peroxide and the high-peroxide PVP batch. We measured peroxide in PVP from both suppliers and found on average twofold higher peroxide in the better performing low-nitrite PVP from supplier B (80 µg/g ± 30 µg/g, n = 7) compared to the high-nitrite PVP from supplier A (40 µg/g ± 22 µg/g, n = 4). Our findings suggest that nitrite and not peroxide in PVP is the key factor that promotes NDMA formation.

More extensive information on nitrite concentrations found in common pharmaceutical excipients is available through a data sharing initiative coordinated by the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium; https://iqconsortium.org and associated database hosted by the non-profit organization Lhasa Limited https://www.lhasalimited.org/Initiatives/nitrite.htm).

3.4. API process optimization at manufacturing site M

The content of residual DMA in the API is an important factor for the formation of NDMA in the drug product, both from a theoretical and a practical point of view. It was known from CPV data that the DMA content in API from site M is higher than in API from site C. DMA was particularly high from approximately the third quarter of 2018 to the first quarter of 2020 (Fig. 10). We had also observed that drug product batches made from API of site M tend to be higher in NDMA than those batches made with API from site C.

For these reasons, a process optimization project was started at site M during the second quarter 2020 with the aim to reduce residual DMA by 50%, i.e., from approx. 0.012% (120 µg/g) to 0.006% (60 µg/g). This was realized by increasing the flowrate of washing water and centrifugation speed for the crystallized crude and finished Metformin, change of the geometry of the spraying nozzle for the washing water, as well as other measures. Since end of the second quarter 2020 residual DMA in API from site M is less variable and in a range of 0.005–0.006% (50–60 µg/g). The maximum allowed content as per Ph.Eur. monograph is at 0.05% (500 µg/g) (EDQM, 2022c).

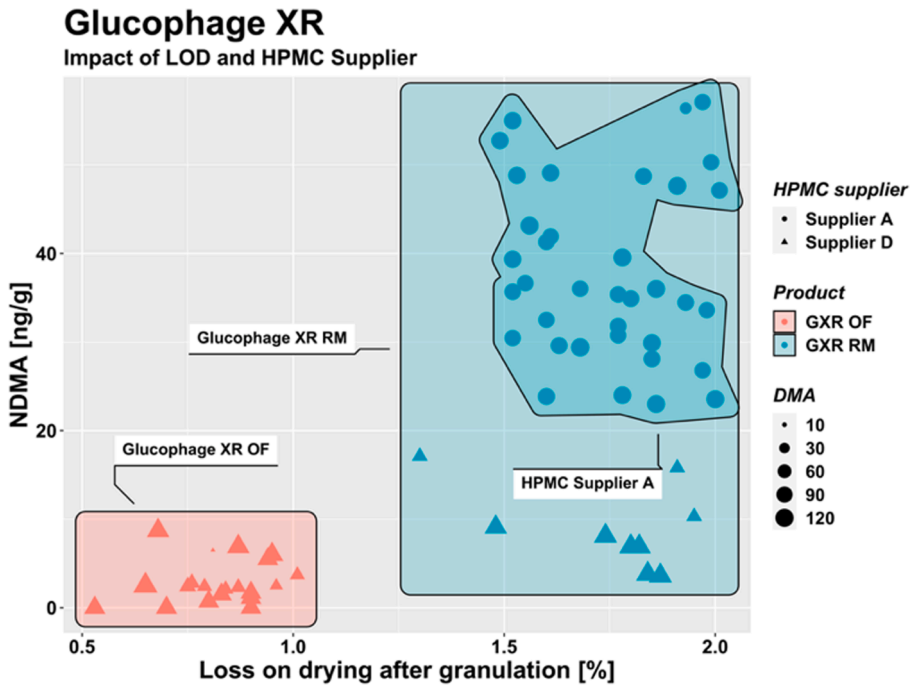

Fig. 8. Impact of loss-on-drying as well as HPMC excipient supplier on NDMA levels in batches of two different Glucophage® XR formulations. The Glucophage® XR "old formulation" (OF) contains 2x the amount of carmellose, a higher percentage of HPMC 100 k and 10% microcrystalline cellulose, compared with the Glucophage® XR reduced mass (RM) formulation shown in Table 1. The reduced mass formulation requires more water to ensure adequate tablet hardness.

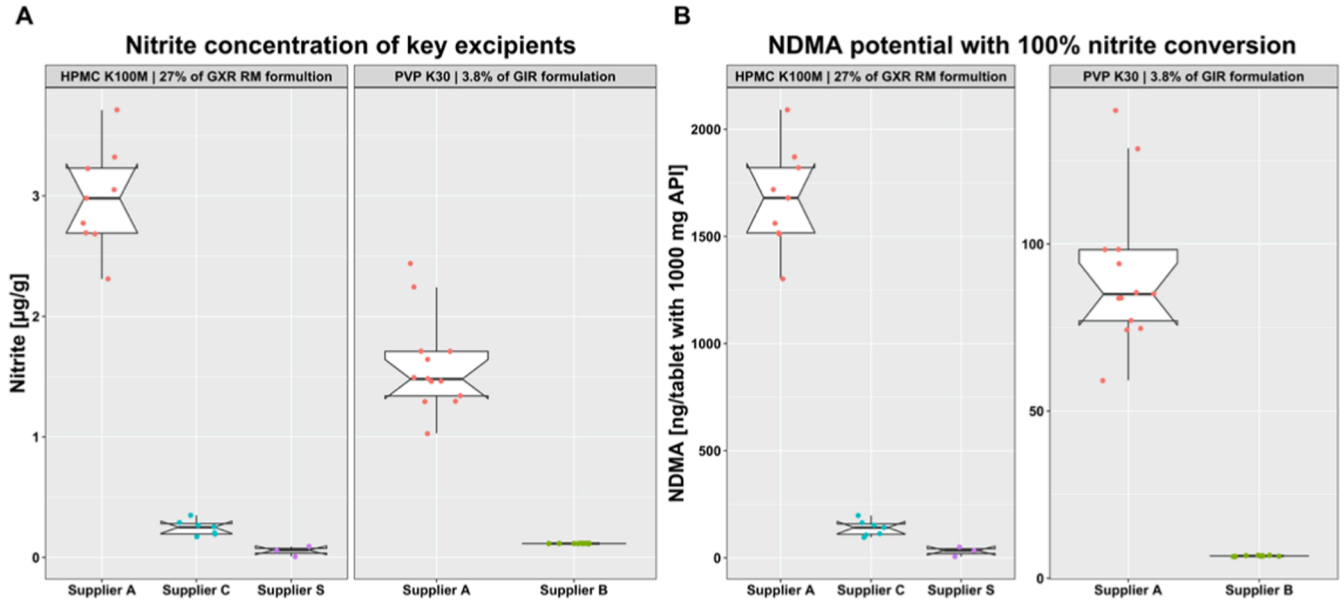

Fig. 9. (A) Nitrite levels in HPMC and PVP batches of different suppliers and (B) maximum NDMA that could be created per 1000 mg strength tablet if 100% of nitrite from HPMC or PVP is converted.

3.5. NDMA data pre and post optimization

The effectiveness of the nitrite optimization measure and DMA reduction was confirmed by continuous monitoring of NDMA in freshly produced drug product batches via implementation of a skip testing program. Respective data is depicted in Fig. 11. It could be confirmed that NDMA was below the ICH M7 control threshold ensuring NMT 30% of acceptable intake (AI) patient exposure (approximately 10 ng/g) since August 2020 with few exceptions managed under the sites' quality systems.

3.6. Designation of a DMA limit to ensure low NDMA in Metformin drug products

For the control of a mutagenic impurity such as NDMA guideline ICH M7 (ICH, 2017) defines a control threshold at 30% of the content that ensures the AI won't be exceeded upon consumption of the maximum daily drug dose.

The NDMA limit was calculated based on the maximum daily dose of Metformin, the acceptable daily intake of NDMA and the product's drug/mass load.

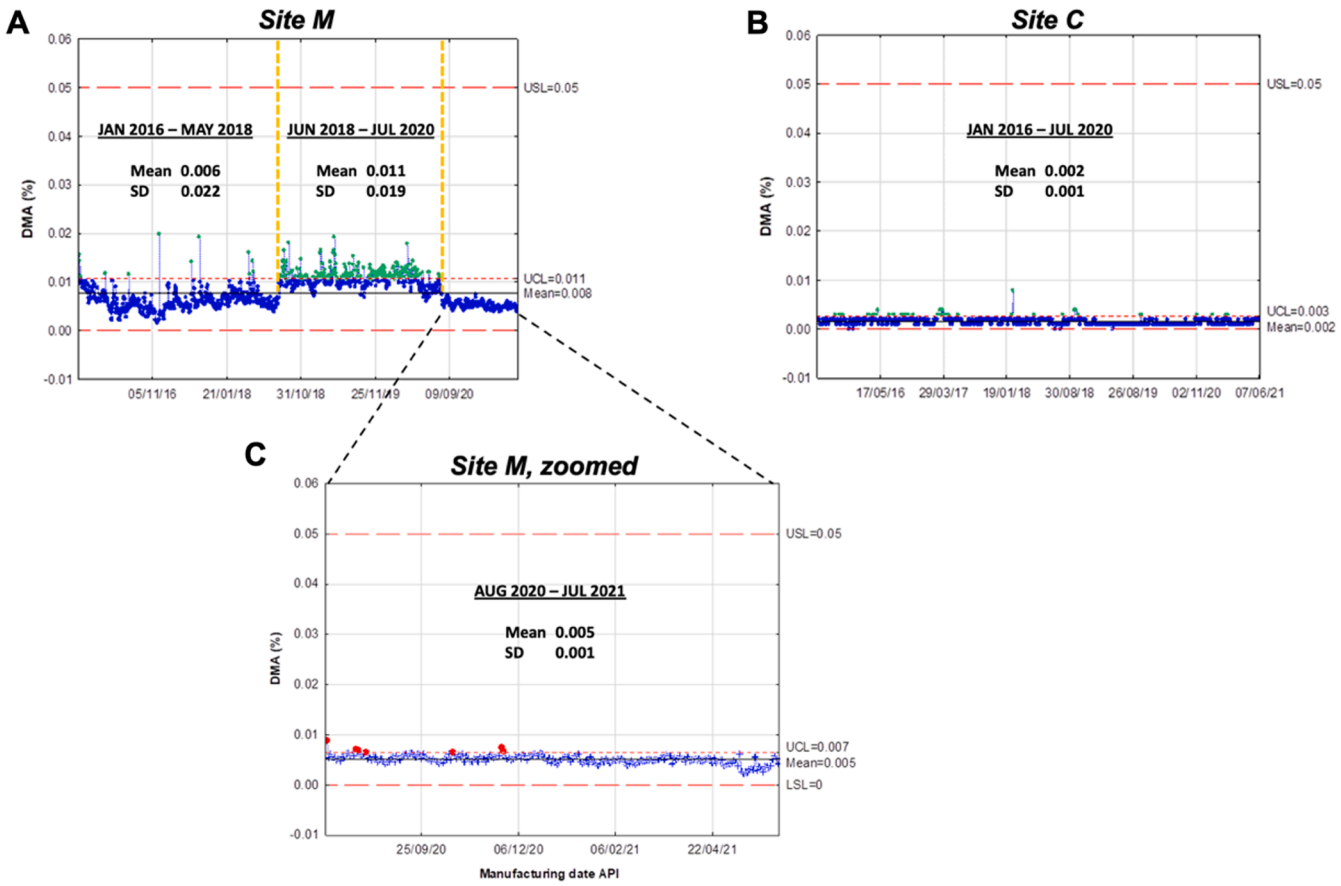

Fig. 10. Control chart of DMA found in API batches produced between **A**) May 2016 and July 2021 at Site M and **B**) May 2016 and July 2021 in Site C. **C)** represents a zoomed view of the period August 2020 – Ocotober 2021.

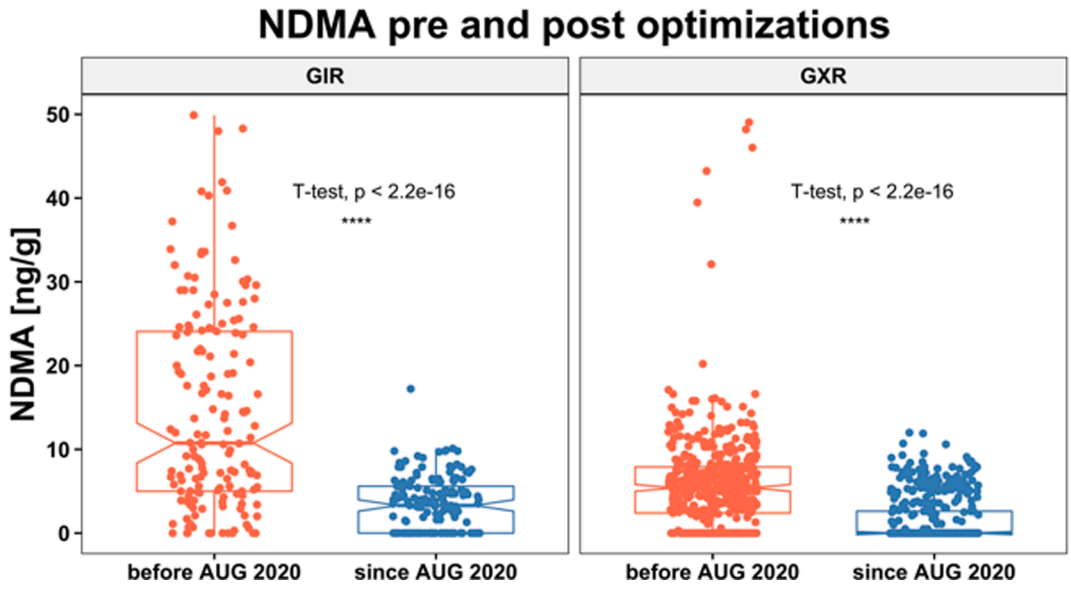

Fig. 11. Comparison of NDMA content measured in Glucophage® and Glucophage® XR manufactured at sites MO and SE before and since August 2021, the date by which DMA-reduced API from site M was available, high-nitrite PVP from supplier A was discontinued at site ME and high-nitrite HPMC was discontinued at site ME. The box plots display NDMA results for 158 Glucophage® batches and 449 Glucophage® XR batches produced before August 2020 as well as 168 Glucophage batches and 516 Glucophage XR batches produced since August 2020.

$$NDMALimit[ppb] = \frac{AI[ng]xDMLx1000}{MDD[mg]}$$

AI NDMA acceptable intake per day = 96 ng.

MDD Maximum Daily Dose (MDD) of Metformin API.

DML Drug/Mass Load = Tablet strength Metformin API [mg] / tablet mass [mg].

To assure that the NDMA content of future batches will be below the control threshold at 30% of that content, a linear regression analysis between API DMA and NDMA content found in drug product was performed. Drug product batches with high-nitrite PVP or HPMC excipient were excluded from this analysis. The DMA content limit is defined by the intersect of the upper bound 95% prediction interval and the NDMA control limit. It is a conservative estimate as the NDMA results for batches with high DMA in the API were retrospectively measured (months-years after the manufacturing), whereas NDMA in batches with low DMA API was measured timely after manufacturing, i.e., batches made with high DMA API were on average older at the time of testing, which may contribute to the higher NDMA values (refer to 3.7). The calculated DMA limit for Glucophage® and Glucophage® XR were 142 µg/g and 60 µg/g, respectively (Fig. 12). As a conservative approach an internal control limit of 60 µg/g will be included in the NDMA control strategy for both drug products. Other drug products may require different DMA limits, depending on their formulation and other properties such as moisture and nitrite content, primary packaging, etc.

3.7. Packaging of Metformin drug products

Cellulose nitrate ("nitrocellulose") is used as a key component/matrix of printing primers, printing inks, and over lacquers commonly used for pharmaceutical packaging materials. The thermal decomposition of cellulose nitrate releases nitrogen oxides (NO_x i.e., NO and NO_2) (Dauerman and Tajima, 1968). Nitric oxide (NO) can react with water to form nitrous acid (HNO_2), a precursor of nitrosating agents as one component. Nitrogen dioxide (NO_2) disproportionates upon reaction with water to yield nitric acid (HNO_3) and nitrous acid (HNO_2) in a 1:1 ratio. It had previously been postulated that the above could lead to the nitrosation of DMA or DEA amine components in the printing ink used on blister lidding foil, which could be transferred to the product through vaporization during heat sealing (EMA/CHMP, 2020). However, to our knowledge, no confirmatory experimental data have been published so far. Here we show NDMA formation in Glucophage tablets caused by nitrocellulose-based ink from the golden full color background of a strip pack aluminum foil. First, we incubated loose tablets removed from strip packs with and without full color background together with the respective emptied strip packs at 60 °C dry heat stress condition in sealed glass vials. In addition, we incubated tablets strip packed in aluminum foil with ("gold") and without ("silver") full color background. The first set-up resulted in a steep linear increase of NDMA in the tablets incubated with golden foil but no NDMA formation in the tablets incubated with silver foil. This can be explained by NO_x created by the decomposition of cellulose nitrate from the golden ink migrating into the tablets, where it caused nitrosation of DMA. Even the second set-up resulted in NDMA formation in combination with the golden foil, even though the tablets were sealed inside the aluminum strip packs, which can be considered impermeable to both cellulose nitrate and NO_x (Fig. 13A). In this case, the NDMA formation is likely to be caused by cellulose nitrate deposition on the inner surface of the foil, which is supplied as a role, i.e., small amounts of cellulose nitrate are likely to permeate through the cellophane layer and attach to the inner low-density-polyethylene (LDPE) layer that comes into contact with the tablets (Fig. 13B). The observed kinetics was not as fast and flattened out towards the end of the storage when all nitrosating agent was consumed. We conclude that nitrocellulose ink is particularly problematic in combination with Metformin products, as they contain DMA as nitrosatable amine component, so that NDMA can be formed easily even if the ink is free from DMA. Using nitrocellulose ink to obtain a full color background represents a worst-case scenario, as the applied amount of nitrocellulose is particularly high.

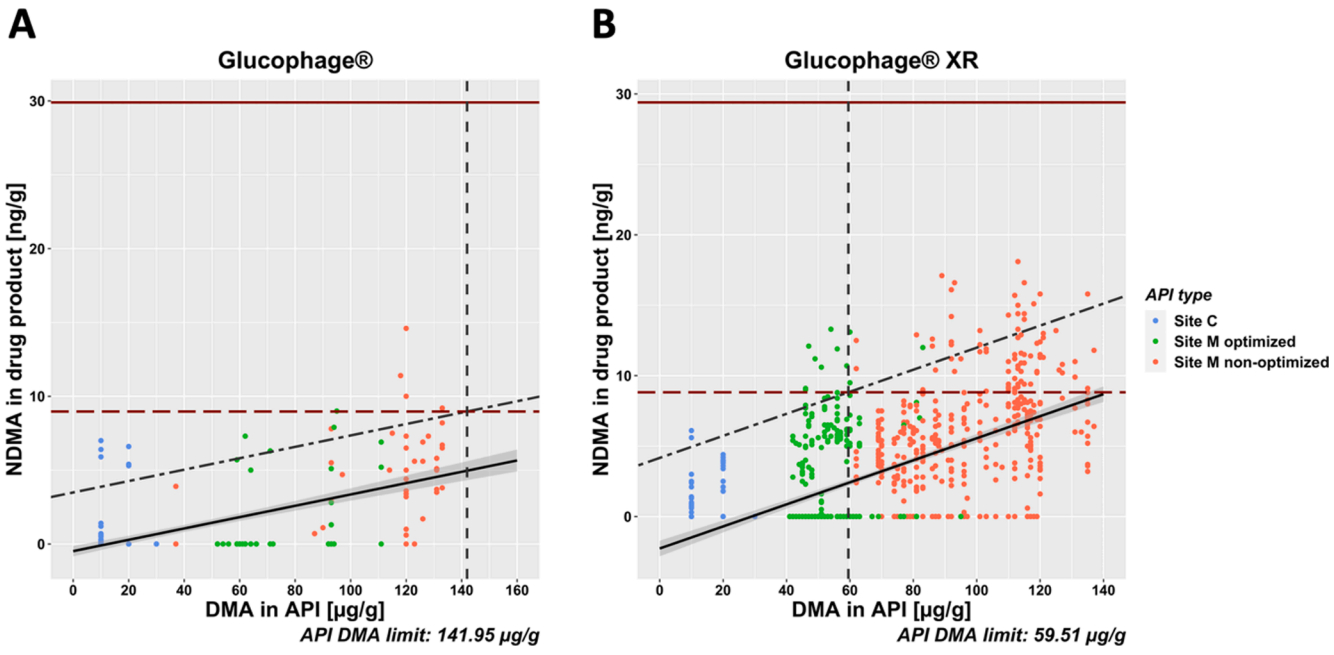

Fig. 12. Determination of DMA limits for (**A**) Glucophage® and (**B**) Glucophage® XR. The regression analysis was performed based on DMA and NDMA data from 276 Glucophage batches and 959 Glucophage® XR batches from the manufacturing site ME. The solid dark red lines represent the maximum allowed NDMA content to ensure that the acceptable intake of 96 ng/day is not exceeded upon consumption of the maximum daily API dose. The dashed dark red lines represent the control threshold at 30% of those concentrations. The solid black lines show the linear regression for the available DMA ~ NDMA results, the dark-grey shading is the 95% confidence interval. The black dot-dashed lines parallel to the regression lines show the upper bounds of the 95% prediction intervals. Black dashed vertical lines were added to illustrate the intersects of upper bound prediction intervals and the NDMA control limits. DMA content in the API not exceeding those at the intersects assures NDMA concent in the DP not exceeding the control limits with 95% confidence. The DMA content corresponding to the dark red control limits is displayed in the lower right corners, respectively.

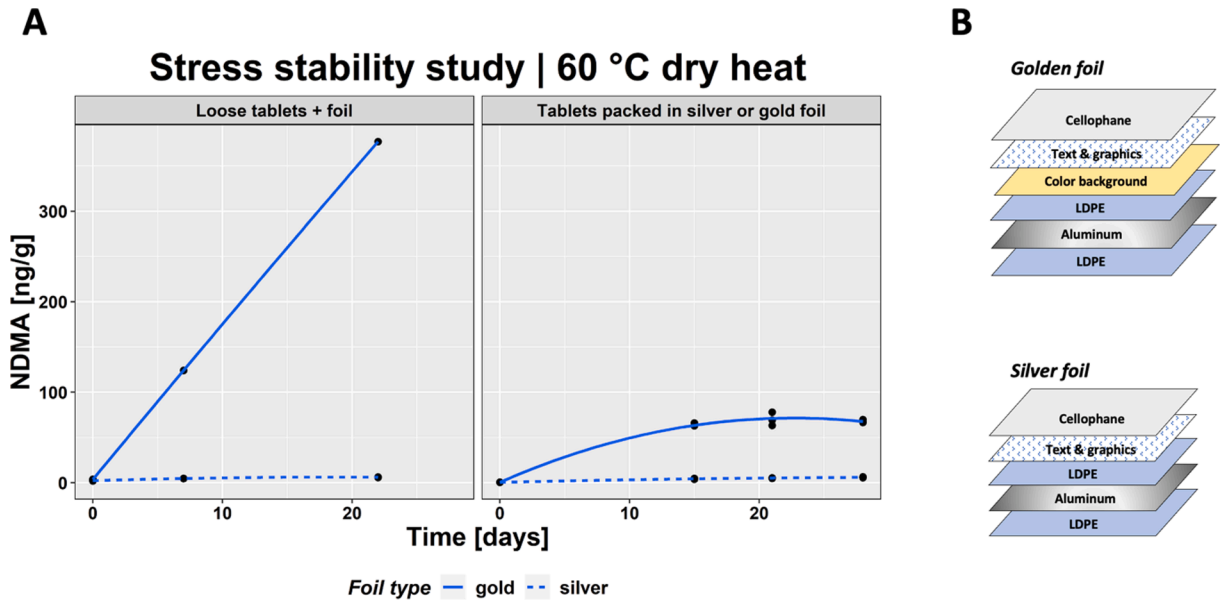

Fig. 13. A) Nitrocellulose-dependent formation of NDMA in Glucophage ®tablets. **B)** Layers of the golden and silver foils used for strip packaging.

3.8. Stability of Metformin drug products

3.8.1. Stress study of Metformin drug products

To investigate whether NDMA can form in blistered drug product we performed a stress study under the conditions 40 °C 75% RH, 70 °C dry heat, and 70 °C 75% RH. Details on the materials included in this study are provided in Table 3. Results of the stability study are depicted in Fig. 14. They are all blister packs in contrast to the strip packs investigated in chapter 3.7. In addition to NDMA content we also measured DMA content at each pull point. Except for batch 3, there was very little NDMA formation at 40 °C 75% RH and little at 70 °C dry heat. The divergent behavior (increased NDMA formation at 40 °C 75% RH and 70 °C dry heat) of batch 3 compared with batch 4, which is the same product, can be explained by the higher nitrite and DMA content (8.5-fold and 16-fold, respectively) at study start. Batch 3 was made with high-nitrite HPMC from supplier A and API from site M, whereas batch 4 was made with low-nitrite HPMC from supplier B and API from site C. By far most NDMA is formed at 70 °C 75% RH. With respect to DMA, no or marginal increase of DMA released from Metformin was detectable at 40 °C 75% RH and 70 °C dry heat. At 70 °C 75% RH, there was a considerable increase of DMA content in the extended-release Glucophage® XR formulations and much less in the immediate release formulations. Obviously, heat in combination with moisture and excipients promotes the release of DMA from Metformin DP (but not API), which causes an increased formation of NDMA at this condition. The NDMA content measured at any time is likely to be a result of both formation rate depending on nitrite, DMA etc. and evaporation rate, depending on the permeability of the packaging material. Glucophage® is packed in PVC blisters, whereas Glucophage® XR is packed in PVC/PVDC blisters, which are less permeable. Our hypothesis is that NDMA can more readily evaporate from a PVC blister compared to a PVC/PVDC blister.

3.9. Analysis of NDMA throughout the Metformin drug product shelf-life

To test whether the findings from the stress study can be translated to more relevant storage conditions, we have set up a long-term ICH stability program. However, as real-time stability data with respect to NDMA formation are still limited (data not shown), we performed a regression analysis of NDMA content measured in tablet batches of different age at the time of analysis. These samples had not been stored in stability chambers but were taken from warehouses or retention sample collections. There is a trend towards higher NDMA values in older batches of Glucophage® and Glucophage® XR RM, but it is so moderate that it does not interfere with the currently assigned shelf-life (SL) of 5 years for Glucophage® and 4 years for Glucophage® XR (Fig. 15).

4. Conclusion

The extensive evaluation of NDMA formation along the API and drug product manufacturing processes presented here has identified key risk factors and enabled the set-up of effective control strategies for NDMA in the studied Metformin pharmaceuticals.

Even though, based on the experience with other APIs with nitrosamine contaminations such as valsartan (EMA, 2018; Sörgel et al., 2019) or ranitidine (EMA, 2020b; King et al., 2020), Metformin API was suspected to be the source of the NDMA found in some Metformin products, it is clearly recognized from our data that this is not the case here. We show that Metformin API is practically free from NDMA if the manufacturing process includes purification through double

Table 3
Production site, packaging material, as well as nitrite, DMA and NDMA content measured before the start of stability storage (T0) for the DP and API batches subject to stress stability testing.

#	Product	Manufacturing site	Nitrite at T0 [ng/g]	DMA at T0 [µg/g]	NDMA at T0 [ng/g]	Packaging Material
1	Glucophage® IR	Site MO	182	104.4	17.1	PVC
2	Glucophage® IR	Site SE	108	91.8	12.5	PVC
3	Glucophage® XR RM	Site SE	460	49.6	27.7	PVC/PVDC 90
4	Glucophage® XR RM	Site SE	54	3.1	6.1	PVC/PVDC 90
5	Glucophage® XR OF	Site SE	99	25.8	8.5	PVC/PVDC 90
6	Metformin Premix	Site M	Not tested	83.0	4.7	N/A

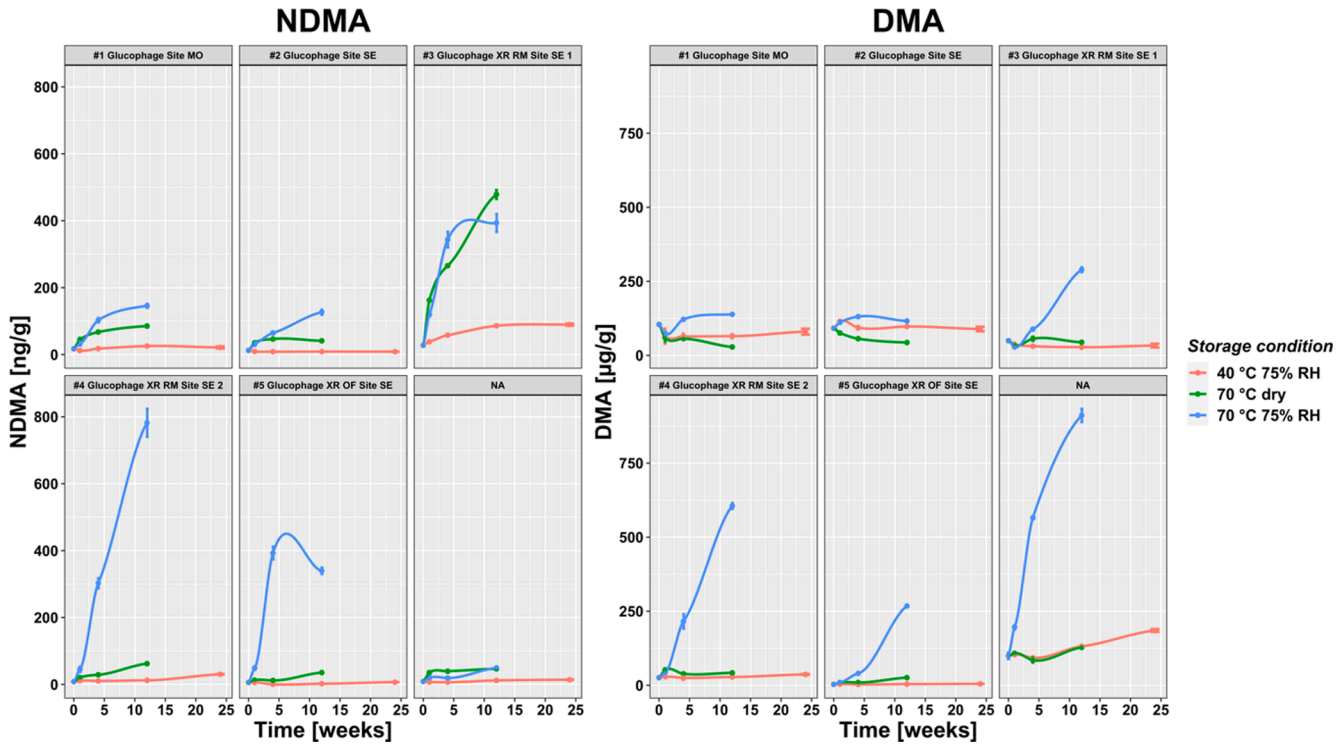

Fig. 14. Metformin DP stress study in blisters. Hot and humid conditions can release DMA from Metformin and promote an accelerated formation of NDMA. All determinations were performed in triplicate.

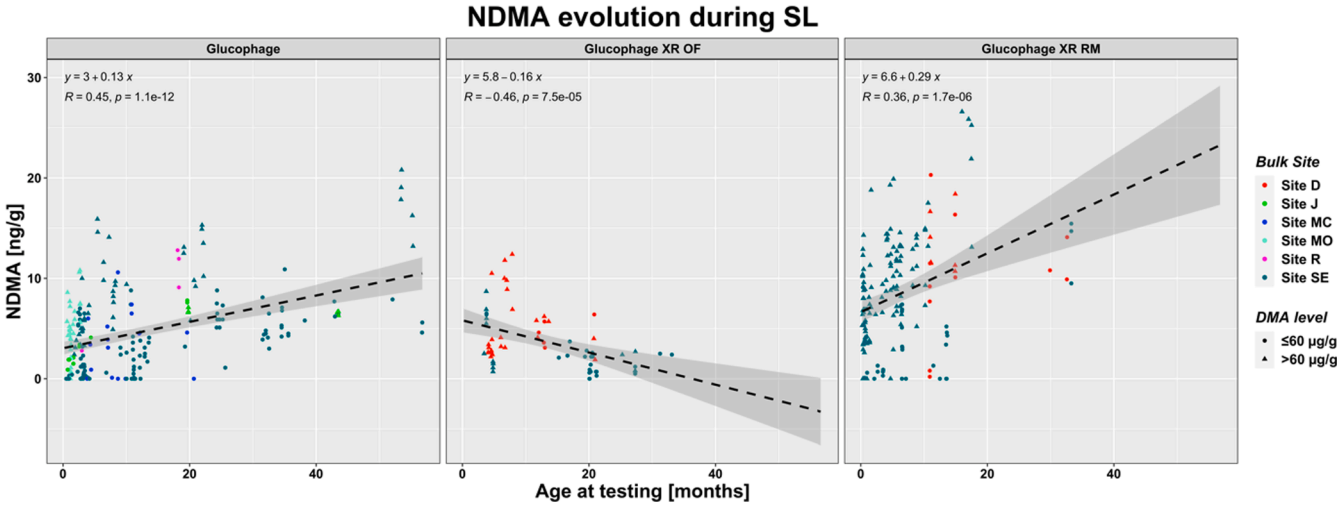

Fig. 15. Level of NDMA vs batch age at the time of testing for the products Glucophage®, Glucophage® XR old formulation and Glucophage® XR reduced mass.

crystallizations. In such case, its contribution to the total NDMA in the product is minimal. The observed NDMA content in the API of<1 ng/g is negligible compared to the appliable limit of ca. 30 ng/g for drug products.

Furthermore, it was demonstrated that NDMA can be created in the drug product manufacturing process, specifically at those steps that introduce nitrite containing excipients and heat. Reduction of residual DMA in the Metformin API in combination with reduction of nitrite introduced by excipients were efficient means to reduce NDMA in the drug products to levels below the ICH M7 control threshold. From a manufacturing perspective, reducing nitrite by changing the excipient supplier is more straight-forward than reducing DMA. It could not be confirmed that peroxide from PVP excipient increases NDMA, whereas the problematic nature of nitrocellulose printing ink was clearly demonstrated through experimental data.

We found a moderate trend towards higher NDMA in older batches in two of the three drug products subject to the analysis, but it was not shelf-life limiting.

Any NDMA control strategy must be tailored to the respective manufacturing process and formulation, as drug products may behave differently with respect to NDMA formation both during manufacturing and shelf-life. Nonetheless, the control of DMA and nitrite will be essential in any case. We used regression analysis to derive drug product dependent limits for the API DMA content that ensure NDMA in the drug product not exceeding the ICH M7 control threshold. These limits are below the current pharmacopoeial limit of 0.05% for Metformin but are technically feasible if the API process includes purification by double crystallization. We cannot recommend a general safe level of DMA as

such level must be determined in a drug product specific fashion. Nevertheless, we believe that the findings presented here will contribute to ensuring patient safety by facilitating continued supply of high-quality Metformin pharmaceuticals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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