WHITE PAPER

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Nitrogen Dioxide Sterilization: Maintains Prefilled Syringe Integrity

Noxilizer has developed an optimal sterilization process specifically for prefilled syringes and other drug-delivery devices. The low temperature NO_2 sterilization process will effectively sterilize the external, gas accessible surfaces of the syringe with minimal to no impact on the contents of the syringe. The process is carried out between 10°C and 30°C, without the need for a deep vacuum, and typically lasts 2 to 3 hours depending upon the product design and packaging materials. The low temperatures and short processing times allow manufacturers to maintain an acceptable time-temperature budget during the manufacturing process. The ability to process without a deep vacuum minimizes the risk of plunger movement on the syringes.

One of the main benefits to the NO_2 sterilization process is that the integrity of the container closure system is maintained for prefilled syringes. This is also true for filled vials and cartridges. The container closure integrity can be demonstrated by testing for NO_2 ingress after a prefilled syringe has been exposed to the NO_2 sterilization process. The NO_2 ingress test is typically performed on syringes that have been filled with water for injection (WFI), deionized (DI) water, or saline solution. If NO_2 were to penetrate the prefilled syringe, either through the rubber plunger, a tip cap or needle shield, it would be absorbed into the aqueous content of the syringe. Upon contact with the liquid water, NO_2 undergoes an equilibrium reaction to form both nitric (HNO_3) and nitrous (HNO_2) acids:[1]

$$2\ NO_2\ (g) + H_2O\ (l) \rightarrow HNO_2\ (aq) + HNO_3\ (aq)$$

$$HNO_2\ (aq) \rightarrow H^+\ (aq) + NO_2^-\ (aq)$$

$$HNO_3\ (aq) \rightarrow H^+\ (aq) + NO_3^-\ (aq)$$

The ingress of NO_2 past the container closure system can be detected by testing for the presence of the anionic components of the acid products, specifically the nitrate anion (NO_3^-). Noxilizer has recently validated a colorimetric assay for $NO_3^-$and nitrite (NO_2^-) anions in solution in order to detect any HNO_3 or HNO_2 products of NO_2 ingress. The assay converts NO_2^- into NO_3^-, and both components are reported as ppm NO_3^-. An example of the chromogenic reaction used to detect NO_2 ingress products is shown in Figure 1, where the DI water blank is colorless, and the sample containing NO_3^- exhibits a bright pink hue. The intensity of the absorbance in the red region, as determined by visible light spectroscopy, is correlated to the NO_3^- concentration in the analyte.

The European Pharmacopoeia (EP) specifies a limit of 0.2 ppm NO_3^- for WFI.[2] Since the US Pharmacopeia does not have a requirement for NO_3^- concentration in WFI, Noxilizer uses the EP limit as a guideline for the acceptability of WFI after the NO_2 ingress test. The limit of detection for the assay is 0.024 ppm NO_3^-, which is an order of magnitude lower than the EP limit. The standard curve showing the response of the assay is shown in Figure 2. Therefore, the assay is sensitive enough to determine if NO_2 ingress has caused the WFI to fall out of EP specification.

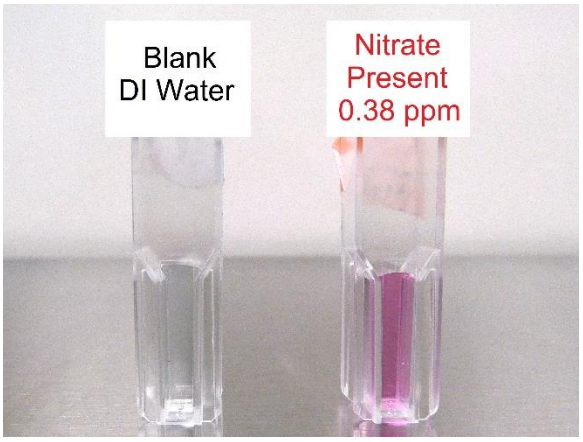

Figure 1. The photograph shows the color change in the WFI upon reaction of the reagents with NO_3^- present in solution.

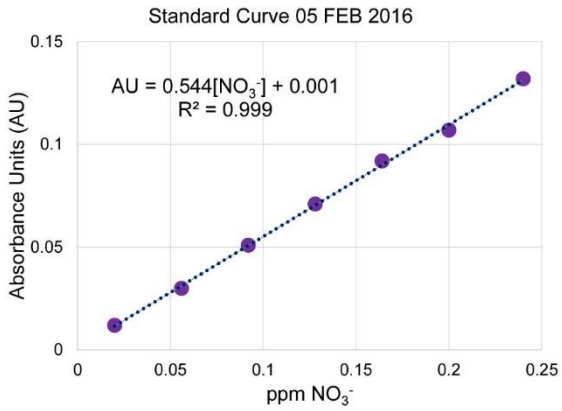

Figure 2. The colorimetric assay can reliably detect NO_3^- anions below the EP limit for NO_3^- in WFI, as demonstrated in the standard curve which spans from 0.024 to 0.240 ppm NO_3^-.

Noxilizer recently demonstrated the integrity of the container closure system in the NO_2 sterilization process using Type 1 glass, 1-mL long syringes with bonded needles. The plunger, which was fitted to a polypropylene rod, was gray pharmaceutical rubber; as was the liner of the rigid needle shield. The syringes were filled with 1 mL of DI water, prior to being packaged in 1073B Tyvek®-Mylar® pouches. The syringes were subsequently exposed to a sterilization process that had been defined following the "overkill approach" in ISO 14937:2009.[3] The process parameters, representative of a typical sterilization process for prefilled syringes, are shown in Table 1.

Table 1. The cycle parameters for the sterilization process are shown. Note that the vacuum refers to the minimum absolute pressure experienced in the sterilization chamber during the process. At sea level the typical ambient pressure is 760 Torr.

Temperature (°C)	Vacuum (Torr)	Relative Humidity (%RH)	NO_2 Dose (mg/L)	Exposure Time (min)	Aeration Time (min)
25 (+/- 3)	700 (+/- 10)	>75	10 (+/- 1)	60 (+/- 1)	90 (+/- 1)

The DI-filled syringes were tested 1 day after exposure to the NO_2 sterilization process. The results of the test for NO_2 ingress are shown in Figure 3. Both the unexposed controls and the exposed syringes remained under the limit of quantification for the assay, demonstrating that the NO_2 sterilization process did not impact the quality of the prefilled syringe contents.

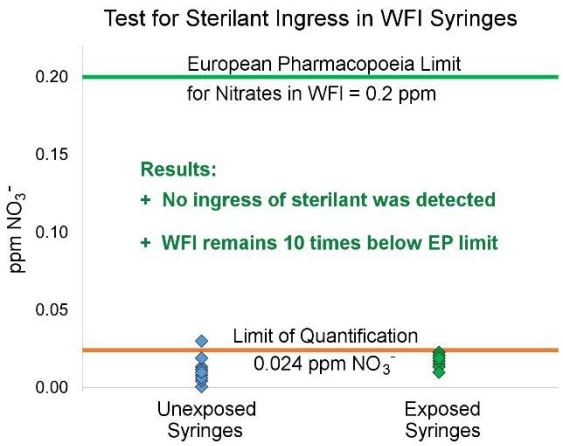

Figure 3. The results of the colorimetric assay demonstrate that the NO_2 sterilization process does not affect the quality of the WFI contents of the syringe.

Noxilizer provides the assay for NO_2 as a means of demonstrating container closure integrity during validation of a sterilization process. It is one piece of information that prefilled syringe manufacturers can use to bolster their regulatory submissions. The data from the assay may be used to supplement any product quality tests, such as purity, activity, or stability tests, that are required for the actual drug or biologic product.

SUMMARY:

NO_2 sterilization provides unique benefits for prefilled syringe and drug-delivery products administered in the operating room or to address other concerns through the manufacturing process. Studies have demonstrated that NO_2 can sterilize the surface of the delivery device without compromising the contents. Further, Noxilizer provides an assay for NO_2 that demonstrates container closure integrity during validation of a sterilization process, supplementing manufacturers' product quality tests and bolstering regulatory submissions.

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REFERENCES:

1. Kameoka, Y., & Pigford, R. L. (1977). Absorption of Nitrogen Dioxide into Water, Sulfuric Acid, Sodium Hydroxide, and Alkaline Sodium Sulfite Aqueous Solutions. *Ind. Eng. Chem. Fund. Industrial & Engineering Chemistry Fundamentals*, 16 (1), 163-169.
2. *European Pharmacopoeia 8th Ed.*, EDQM, European Pharmacopoeia: Council of Europe, Strasbourg. Monograph 0169, *Water for Injections*.
3. ISO 14937:2009 – Sterilization of health care products – General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices. International Organization for Standardization, Geneva, Switzerland.