Oxygen Headspace Analysis for Air Headspace to Develop and Validate Container Closure Integrity Methods

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Abstract

Container closure integrity testing (CCIT) by laser-based headspace oxygen can be performed for samples with an air headspace for the detection of both small (≤20 micron) and large (0.5-2 mm) defects, evaluating the container closure integrity (CCI) of a sample at any point in the product-package life cycle. This article will discuss developing and validating a CCI method using oxygen headspace analysis for air headspace samples, including considerations for different container sizes and fill volumes.

Introduction

Container closure integrity testing (CCIT) by laser-based headspace oxygen can be performed for samples with an air headspace by storing the air headspace samples in a sealed nitrogen environment and monitoring for a decrease in headspace oxygen. This non-destructive, quantitative, deterministic test allows for the detection of both small (≤20 micron) and large (0.5-2 mm) defects, evaluating the container closure integrity (CCI) of a sample at any point in the product-package life cycle. This article will discuss developing and validating a CCI method using oxygen headspace analysis for air headspace samples, including considerations for different container sizes and fill volumes.

CCIT can be useful at multiple points during a product-package lifetime. One common reason to perform CCI testing is in lieu of sterility testing (Food and Drug Adminstration, 2008). For many drugs, such as parenteral drug products, maintenance of sterility is a key quality attribute. To utilize CCIT to establish the sterility quality attribute, traditional sterility testing or another validated sterility release mechanism is performed at release or the initial time point to verify the product batch is sterile. Then, at a future stability timepoint (typically annual) CCIT can be performed in lieu of sterility to establish there are no defects through which microbiological contamination could occur.

The use of laser-based headspace analysis, including oxygen headspace analysis, for container closure integrity testing is listed in the USP <1207> Package Integrity Evaluation – Sterile Products Chapter. The method development and validation in this article follows the guidance listed in the USP <1207> chapter. (United States Pharmacopeial Convention, 2016).

Oxygen Headspace Analysis for CCIT

Intuitively, oxygen headspace analysis can be used to detect a leak in containers that starts with an altered headspace (e.g., purged vials, lyophilization vials stoppered under nitrogen, etc.). If there is a defect in the container, the closure or the interface between the two, the oxygen level in the container will increase over time. Monitoring this increase compared to positive and negative controls can allow for the determination of container closure integrity. However, many products are stoppered with a simple air headspace instead of an altered headspace.

Oxygen headspace analysis also can be used to determine CCI for samples with an initial air headspace by deliberate exposure to a low oxygen/high nitrogen environment. If there is a breach in CCI, these

challenge conditions will create a detectable decrease in headspace oxygen. Typically, samples are first measured for an initial oxygen concentration (around 20% atmosphere oxygen). The samples are then placed in a vessel that is purged with nitrogen and sealed. Alternatively, a glove box or similar container with a continuous nitrogen purge could be used to create the challenge conditions. After storage in nitrogen for a predetermined length of time, the samples are removed, and the headspace oxygen concentration is measured a second time. The change in headspace oxygen is calculated for each container and compared to a maximum allowable oxygen change. The method development and validation information that follows will focus on the case of samples with an initial air headspace.

Method Development

When developing a headspace oxygen CCIT method for air samples, four interdependent parameters must be determined: 1) the range of defects to be detected (both small and large); 2) the appropriate challenge time in nitrogen for the samples; 3) the post-challenge testing window for removal from the challenge conditions and measurement of the samples; and 4) the maximum allowable oxygen change (MAOC), which is the maximum permissible change above which the CCI should be considered compromised.

One factor that has a significant impact on the preceding parameters is the volume of the headspace. When samples are stored in challenge conditions, headspace gas concentrations change through the process of diffusion. When the vial headspace contains air and is placed in a high nitrogen/low oxygen environment, the total pressure inside and outside the container are equal, but there is a partial pressure differential for the oxygen and nitrogen concentrations. Over time, the partial pressure difference will equilibrate through any defects resulting in a measurable decrease in headspace oxygen concentration inside the vial. For a larger headspace, more oxygen and nitrogen will need to diffuse through the defect to create an equivalent change in a container with a smaller headspace. In other words, larger headspaces have slower changes in headspace oxygen over time. One impact of this is that if the liquid fill volume for a container is changed, that also can impact the method parameters for successful detection of the desired defect range.

The first of the four parameters we'll be outlining is the range of defect sizes to be detected. Current guidance has indicated that 20 microns or less is the expectation for the defect detection limit for CCI methods. Our default initial small defect used is a 5-micron defect laser drilled through the glass wall of the container. However, depending on the container size and client requirements, defects as small as 2-microns or as large as 20 microns have been used for the lower end of the defect range. For the higher end of the defect range, we commonly create defects in one of two ways. The first is to use a biopsy punch (e.g., 2mm punch) to bore a defect in the stopper, removing material and leaving behind a large defect. The second route is inserting a needle (e.g., 0.5mm inner diameter, 21-gauge, 1-inch disposable needle with luer lock removed) in the stopper and leaving it in place for the duration of the test.

The second parameter determined in method development is challenge time. To detect a small defect, the challenge time can be extended until it becomes detectable. As part of method development, we perform a run with positive and negative controls and measure them at multiple time points, returning them to the nitrogen challenge conditions between measurements to determine the best challenge time to detect the desired smallest defect. Depending on the headspace volume, five-micron defects commonly can be detected after six to 48 hours exposure to nitrogen challenge conditions. Our most common challenge time is 24 hours, allowing a single analyst to measure the initial and final time points at the same time on two consecutive days.

The third key parameter to discuss is the post-challenge testing window, which is a validated window of time after removal from challenge conditions that all positive controls remain detectable. This is a limiting factor for the large defect end of the defect range as it allows for the fastest headspace oxygen change. Short of placing the headspace oxygen analyzer in a nitrogen-purged glove box, it is necessary to remove the samples from nitrogen prior to measurement. After their removal from challenge conditions the samples will continue to diffuse with the environment. If left out of the challenge conditions too long, all samples will measure similarly to initial oxygen concentrations regardless of the presence of defects. Therefore, it is important to determine and validate a post-challenge testing window within which the full defect range change be detected. For large defects in containers with smaller headspace volumes, the container can equilibrate to its surroundings in less than 20 minutes. While each individual headspace oxygen measurement is very quick (5-10 seconds), the logistics of removal and measuring with correct label identification can take time, particularly for larger numbers of samples. For that reason, we typically suggest a post-challenge testing window of at least 30 minutes (30 minutes to two hours typically targeted for post-challenge testing window). If a defect cannot be detected 30 minutes after removal in method development, a smaller defect would be chosen for the top end of the defect range.

The fourth key parameter in developing a CCIT method is the MAOC, which serves a similar purpose as the maximum allowable leakage limit discussed in USP <1207> (United States Pharmacopeial Convention, 2016). This is a product-package configuration specific value that is used to differentiate between leaking and non-leaking packages. When the headspace is air, the MAOC would be a maximum decrease in headspace oxygen. The MAOC needs to be greater than the change observed in non-leaking packages and less than the change observed in leaking packages. Changes observed in non-leaking packages typically are due to instrument noise. For tests with longer challenge times, permeation also needs to be considered. Permeation through the components is a source of gas ingress in sealed packages not due to a defect. To quantify the scale of change due to permeation, negative controls can be included in the method development test runs. However, for the 48-hour or shorter methods typically performed for this test, change due to permeation is not typically visible compared to instrument noise.

Instrument noise should be considered when setting the MAOC. For laser-based headspace analysis, noise is affected by both the laser path length through the headspace and the laser power after passing through the headspace. The laser path length is the inner diameter of the container at the point where the laser passes though the container. The larger the diameter of the container, the better the standard deviation of repeated measurements. The exception to this is if the container also decreases the laser power, which, if decreased by two-thirds or more of total power, can increase standard deviations of repeated measurements. Common reasons for a significant power decrease would be powder/lyophilization fragments on the walls of the vials or amber vials. Many other causes of measurement error are largely negated by taking initial and final measurements of a specific vial and comparing the change for that specific vial. This allows for any variation due to overall path length, vapor pressure or other variables that might impact the oxygen measurement to be consistent for both timepoints and have less impact on the calculated change.

In addition to the measurement precision (standard deviation), an additional factor to consider when setting your MAOC is compounding error, which can occur due to small variations in the calibration. To account for these and because we can easily increase the oxygen change in the positive controls by increasing the challenge time, we recommend using 10 times the maximum standard deviation over the measurement range as a minimum value for the MAOC.

As part of method validation, repeated measurements of test samples and oxygen standards (those used to calibrate and establish system suitability of the instrument) are used to demonstrate the capabilities of the instrument to measure the desired package configuration. Similar measurements can be taken as part of method development to establish acceptance criteria for the validation.

Method validation

To substantiate an oxygen headspace method, we validated both the ability of the instrument to correctly measure the headspace oxygen in the desired container and the ability of the method to differentiate between positive controls with defects and negative controls without defects (i.e., differentiate leaking and non-leaking packages).

To validate the capabilities of the instrument, acceptance criteria should first be established for precision, accuracy, linearity and intermediate precision. As part of the validation, an analyst measures the oxygen standards and a test sample 10 times each, which are then used in calculations to establish the validation parameters. To determine precision, the standard deviation of the n=10 measurements of test samples and oxygen standards can be used. For linearity and accuracy, the measured values of the oxygen standards can be compared to the known oxygen concentrations to calculate linearity and accuracy. For intermediate precision, the above is repeated by a second analyst to establish intermediate precision with additional acceptance criteria on the calculation of the standard deviation of n=20 measurements (10 per analyst).

To thoroughly establish the ability of the method to detect the desired defect range, the validation is structured to include three CCI runs with positive and negative controls. The positive controls include defects at both ends of the defect range established in method development. Negative controls can include both sample vials and controls prepared by the same method as the positive controls if different from the sample vials. To establish method specificity and robustness, an analyst performs two runs, one each at the minimum and maximum challenge time. The minimum challenge time run also includes additional measurements of the positive controls to validate the post-challenge testing window. To establish intermediate precision for the method, a second analyst performs a single run at the minimum challenge time on a different day (and instrument if available) than the first analyst.

Conclusion

The method development and validation described in this article can be used to generate a CCIT method for any container with a measurable headspace with an initial air oxygen concentration. The validated test method then can be used to establish container closure integrity throughout a product life cycle. Performed in lieu of sterility at later stability time points, the samples can be quickly and non-destructively tested for defects over a wide defect range, allowing for the samples to be reused for further testing.

References

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