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ARTICLE

Test Method Development for a Container Closure Integrity Test Using Headspace Gas Ingress

CONTAINER CLOSURE INTEGRITY

To ensure patient safety, good container closure integrity (CCI) is of great importance for all sterile injectable products. Good CCI prevents ingress of contaminants, such as microorganisms and gases, that can negatively affect product sterility and/or efficacy. Not all defects that can cause a loss in CCI can be detected using visual inspection processes. A microscopic crack in the glass might be hidden underneath the aluminum crimp seal for example. Or a container has only temporarily lost closure and now appears to be sealed properly, even though an undesirable exchange between the sterile product and the non-sterile environment has occurred. CCI testing is therefore needed, to ensure that the closure of a product package has not been compromised.

Recent regulatory guidance has made clear that thereis no 'gold standard' for CCI testing. Selection of an appropriate CCI test method should depend on the specific container closure system, the stage in the product life cycle, and the testing throughput required. Chapter <1207> of the United States Pharmacopeia (USP) describes various available CCI testing techniques, and provides guidance on method development and validation in various stages of the product life cycle. Headspace analysis (i.e., analysis of the region above the product) is described as a deterministic CCI test, which is preferred over the use of probabilistic methods such as blue dye ingress and microbial challenge testing (1),(2).

As a CCI test method, headspace analysis is based on detecting changes in the headspace gas composition that result from gas ingress through a leak. Non-destructive headspace analysis, using laser-based spectroscopy, can be used to directly quantify the gas concentration inside a sealed parenteral package. It can be applied to a range of product configurations and formulations, and has historically been used for detecting leak defects in a modified headspace product.

HEADSPACE GAS INGRESS TESTING

For sterile products that are prepared with a modified headspace, a leak will naturally result in the ingress of air from the surrounding environment. A measured increase of headspace oxygen concentration will therefore imply a loss of CCI. However, when a container has a headspace already containing atmospheric air (i.e., a non-modified headspace), a leak does not naturally result in a change in headspace gas composition. For this situation Lighthouse has developed an alternative approach for CCI testing that is based on the ingress of carbon dioxide as a tracer gas. Because very little carbon dioxide is naturally present at atmospheric conditions, it is a reliable option as even small increases in concentration can mark an integrity failure.

By placing a container with an air headspace in a carbon dioxide-rich environment, the sample can be pre-conditioned prior to headspace analysis.

When measurements reveal increased levels of carbon dioxide in the headspace of the sample, gas has been able to ingress into the container, meaning the containers has lost CCI.This approach is very similar to the probabilistic blue dye ingress method in concept but has the significant advantages of being deterministic, rapid, non-destructive, and analytical.

For pre-conditioning of product packages, Lighthouse has designed a CCI test vessel (Figure 1). Inside the test vessel an environment can be created that accelerates gas ingress through a leak defect. Various conditioning cycles can be used, including pulling an initial vacuum and backfilling with carbon dioxide and/or overpressurizing with carbon dioxide, to allow fast gas ingress.

METHOD DEVELOPMENT

The method of headspace gas ingress testing is deterministic and analytical and can therefore be robustly developed and validated. During method development it is important to take into account the scope of the method:

- In which stage of the product life cycle is the test going to be used?
- What type of defects have the highest risk of occurring?
- What leak detection sensitivity is required?
- Can the product formulation interfere with the CCI test?
- Is the package or product affected by the test method?

Figure 1: CCI test vessel and carbon dioxide headspace analyzer, used in the study described in this article.

POSITIVE CONTROLS

During method development for a specific product-package configuration, method parameters are determined and tested to ensure the CCI testing method can differentiate between leaking and non-leaking packages.

Various negative controls (packages without an intentional defect) and positive controls (packages with an intentional defect) are tested during this development stage. Positive controls should be selected according to the scope of the method, taking into consideration the purpose of the positive control. Examples of various positive controls, including their advantages and disadvantages, are listed in Table 1.

Real world defects are usually very complex, with no defined diameter, length or shape and some defects can even change over time. For instance, using micron-sized wires between a vial neck and a stopper (Figure 2, left image) would simulate a fiber or a hair stuck in the sealing area, which is a realistic defect.

However, the actual leak path resulting from the wire is not well-defined. It can vary from sample to sample depending on the sample preparation, the elasticity of the rubber stopper and the crimping compression (3).

Other positive controls, such as a micro-capillary placed into a rubber stopper (Figure 2, right image), have a defined diameter and path length which can give insight into leak detection sensitivity. However, these defects are not likely to occur in real product packages.

Figure 2: Left) Example of a positive control where a microwire is placed between the vial neck and the stopper. Right) Example of a positive control where a microcapillary is placed into a rubber stopper.

Positive control type	Advantages	Disadvantages
Laser-drilled defects in container wall	- Relatively well-defined leak path - Easy to use	- Expensive - Ordering lead time
Micro-capillary	- Defined leak path - Cheap	- Unrealistic defect - Sample preparation dependent (cutting of tube)
Micro-pipette	- Defined leak path - Possible to prepare sub-micron defects	- Unrealistic defect - Labor intensive sample preparation - Handling (breakage of tip)
Needle puncture through rubber	- Defined leak path - Easy to use - Cheap	- Unrealistic defect
Micron-sized wire in sealing area	- Realistic defect - Cheap	- Undefined leak path - Very dependent on package material and process
Micro-cracks	- Realistic defect	- Undefined leak path

Table 1: Overview of positive controls used in CCI test method development including examples of their advantages and disadvantages.

EMPTY AND PRODUCT FILLED 15R VIAL

This article further describes a study that illustrates the use and behavior of different positive controls for a 15R vial. In this study, laser drilled vials and microwires placed between vial neck and stopper are used as positive controls. The change in headspace carbon dioxide levels, which would indicate a loss in CCI, are analyzed using a Lighthouse Instruments Headspace Carbon Dioxide analyzer.

In addition to the empty positive control set, two different sets of liquid filled positive controls are added. One set consists of vials filled with 5 mL of a phosphate buffered saline (PBS) solution, the other set consists of vials filled with 5 mL of Bovine Serum Albumin (BSA) in PBS (1 mg/mL). The laser-drilled defects were positioned underneath the liquid fill. For the micro-wire defects, the vials were inverted three times prior to performing the CCI test to ensure the fill came into contact with the defect. For laser-drilled positive controls where the defect is submerged underneath the liquid fill, detection of the loss in CCI will generally be more difficult.

For successful detection, the carbon dioxide tracer gas would first have to overcome the liquid's surface tension before reaching the headspace.

What makes the detection of a loss in CCI in liquid filled product packages even more challenging is the tendency of liquid products, and protein-based solutions in particular, to clog defect sites. As before, the study also includes a set of negative controls.

Prior to conditioning the samples with the tracer gas, a baseline headspace measurement (T_0) is made to confirm no significant amounts of carbon dioxide are present in the vials. The various positive and negative control sets are then placed in the CCI test vessel and undergo a carbon dioxide overpressure cycle for 30 minutes. After this pre-conditioning step a headspace carbon dioxide measurement (T_1) is made.

Figures 3 and 4 show the measured carbon dioxide content at T_0 and T_1 in the laser-drilled and microwire positive control sets, respectively. The results show that all empty laser-drilled positive controls are easily and consistently detected through significant increases in carbon dioxide after the 30 minutes pre-conditioning cycle.

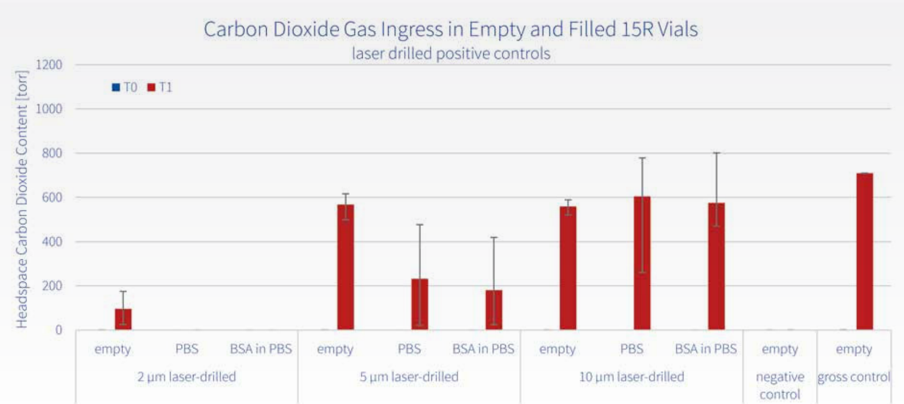

Figure 3: CCI Test results with laser drilled positive controls. Headspace carbon dioxide ingress into 15R vials before (T_0) and after (T_1) a pre-conditioning cycle inside the CCI test vessel. Laser-drilled positive control vials were either empty, filled with PBS, or filled with 1 mg/mL BSA in PBS. Values show averages of five measurements on ten laser-drilled positive control vials and five measurements on three vials for the negative and gross positive controls. Error bars show the min max range across the averages for each sample set.

For the liquid filled laser-drilled positive controls, the outcome is slightly different. All positive control sets with laser-drilled defects of at least 5 µm are consistently and easily detected using the 30 minutes pre-conditioning cycle. As anticipated by the tendency of liquid products to clog defects however, the 2 µm laser-drilled do not reveal significant increases in headspace carbon dioxide.

For the microwire positive control sets, a larger sample-to-sample variation within sample sets can be observed. This variation is a good illustration of the realistic nature of microwire defects as the compressed rubber stopper will flow and partially seal around the wire.

Overall, it can be said from these measurements that the larger the microwire the higher the chance of a defect being detectable regardless of the fill of the sample. It is important to note that these smaller defect sizes do not represent the limit of detection of the methodology. Both the overpressure and hold time can be easily adjusted to ultimately identify the desired defect size for this, or any other, product-package configuration.

METHOD VALIDATION

When validating a final CCI test method, it should be demonstrated that the method is able to accurately differentiate between leaking and non-leaking containers and to verify the Limit of Detection (LOD). This again requires the use of positive and negative controls. The determined CCI test method parameters, which are extensively tested during method development, are used as part of a defined test method during the validation. The test is performed multiple times, with multiple operators, to verify that the selected positive controls (representing a critical defect) are detected as leaking samples, and the negative controls are passed as having good CCI.

In general, the gas ingress CCI test, using a tracer gas and a CCIT vessel, is capable of generating robust analytical CCI data in all stages of the product life cycle. It is a clear improvement to the traditional blue dye ingress test, using a 'bath' of tracer gas instead of liquid dye for increased sensitivity and making an analytical measurement of the gas ingress through a leak.

The non-destructive nature of the test and the generation of analytical data enables further robust method development of CCI test methods. These methods can be optimized per product configuration and then validated with scientific studies, demonstrating the detection of critical leaks and the determination of limits of detection.

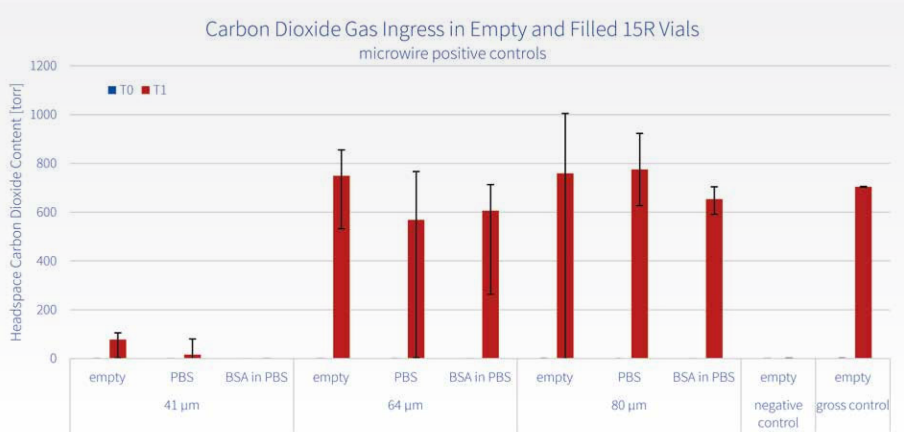

Figure 4: CCI Test results with microwire positive controls. Headspace carbon dioxide ingress into 15R vials before (T_0) and after (T_1) a pre-conditioning cycle inside the CCI test vessel. Microwire positive control vials were either empty, filled with PBS, or filled with 1 mg/mL BSA in PBS. Values show averages of five measurements on ten microwire positive control vials and five measurements on three vials for the negative and gross positive controls. Error bars show the min max range across the averages for each sample set.

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