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ARTICLE

Implementing USP <382>: Evaluating Elastomer Resealing Capacity with Non-Destructive Headspace Analysis

Introduction

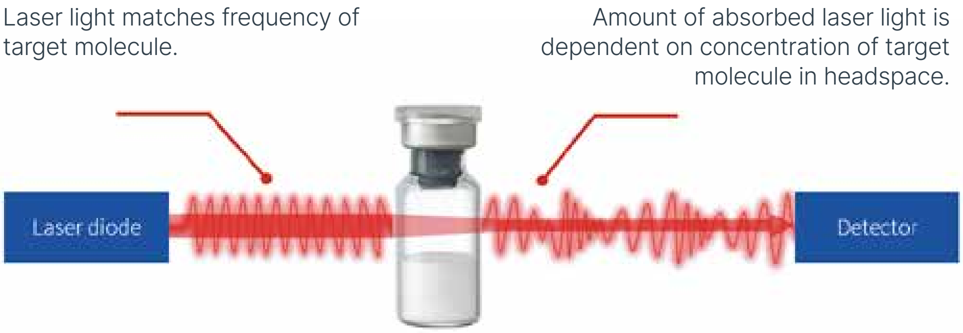
On December 1, 2025, USP <382> “Elastomeric Component Functional Suitability in Parenteral Product Packaging/Delivery Systems” became officially effective, replacing the functionality testing portions of USP <381>.

Historically, elastomeric components like rubber stoppers were tested in isolation by the supplier to ensure basic material compliance. However, the new USP <382> requires a holistic "systems approach". It mandates that the entire assembled packaging/ delivery system—including the container and any elastomeric seal—must be evaluated for its "fitness-for-intended-use".

With regard to container closure integrity testing, one of the most significant changes required by USP <382> applies to multi-dose systems. When a health-care professional or patient pierces a multi-dose vial multiple times, the rubber stopper must consistently reseal to prevent microbial ingress and product loss.

Under the old USP <381> section 4.3.3 “Self-Sealing Capacity”, this was evaluated using a probabilistic "blue dye" immersion test. Today, USP <382> section 5.2 “Needle Self-Sealing Capacity” explicitly points to USP <1207>, demanding the use of sensitive, deterministic methods to prove that the package maintains its Maximum Allowable Leakage Limit (MALL) after repeated punctures.

One of the deterministic methods referenced in USP <1207> is laser-based headspace gas analysis, a non-destructive technique that detects defects by measuring changes in the gas composition within a container’s headspace (Figure 1). While its sensitivity is comparable to helium leak testing, headspace analysis offers the advantages of being non-destructive and that samples can be conditioned and analyzed in batches. Furthermore, headspace CCIT methodologies can be used across the full lifecycle of the product, from testing empty containers for inherent package integrity to testing product-filled samples for stability studies.



Laser light matches frequency of target molecule.

Amount of absorbed laser light is dependent on concentration of target molecule in headspace.

Non-destructive (product not lost, sample can be re-measured)
Rapid (~ 1 sec)

Figure 1 — Schematic representation of non-destructive laser-based headspace analysis. The headspace gas concentration of interest is directly measured within seconds.

A common example of a change in headspace gas composition resulting from a loss of container closure integrity (CCI) is a change in pressure. A typical product where this can occur are freeze-dried pharmaceuticals, which may be filled under reduced pressure to facilitate reconstitution.

When CCI is compromised the pressure equilibrates with the surrounding environment. This change can be directly detected using laser-based headspace pressure measurements.

Another example involves pharmaceuticals filled under an inert nitrogen atmosphere such as oxygen-sensitive products. A loss of CCI in these systems leads to ingress of ambient air, which is measurable as an increase in headspace oxygen levels.

Both approaches to detecting a loss of CCI are demonstrated in this article. It is important to note, however, that a modified headspace—such as reduced pressure or an oxygen-free environment—is not a requirement for performing CCI testing using non-destructive laser-based headspace analysis. A general approach using carbon dioxide as a tracer gas can be validated in this case and is particularly useful for both inherent package integrity and routine CCI testing. This will also be discussed further in this article.

Evaluating Stopper Resealing Performance Using Headspace Gas Analysis

To demonstrate the suitability of headspace gas analysis for evaluating stopper resealing performance, a study was conducted using 2mL and 20mL glass vial configurations with crimp-sealed elastomeric stoppers.

For each vial configuration, corresponding controls and test samples were prepared. The different types of controls and test samples were used to evaluate both method performance and stopper behavior:

METHOD PERFORMANCE				STOPPER BEHAVIOR
Micron-sized positive controls containing certified 5 µm laser-drilled defects	**Gross positive controls** created by inserting a syringe needle through the stopper which remained during the CCI test	**Unmodified controls** representing intact packaging systems	**Negative controls** intact packaging systems that were not exposed to the conditioning environment applied to the other control and test samples	**Test samples** sealed with a headspace of either nitrogen or vacuum, were intentionally punctured multiple times with syringe needles to simulate real-world use of multi-dose vials

The test samples were prepared under either nitrogen or vacuum conditions to mimic different pharmaceutical processing scenarios, including lyophilization. Having a modified headspace at the time of sealing the vials, allowed the study to also evaluate real-time dynamics of stopper resealing during repeated needle punctures.

Real-time Stopper Resealing Dynamics

In multi-dose vial applications, elastomeric stoppers must reseal after repeated needle punctures to regain container closure integrity. Although the procedure in USP <382> section 5.2 describes that each punctured closure should be tested after completing the final puncture, it was decided to explore the elastomer resealing during the puncturing protocol as well.

Test samples were prepared with a modified headspace and punctured with syringes. The plunger of each syringe was fully depressed to reduce exposure to ambient air that could occur if the needle were left open. The change in headspace conditions – indicative of a loss in CCI - was monitored during the puncturing protocol.

TEST SAMPLES WITH A NITROGEN HEADSPACE

These samples mimic a pharmaceutical manufacturing process typically used for oxygen sensitive products. Initial measurements confirmed that the samples were prepared with very low oxygen levels. After puncturing the stopper and removing the needle, the samples were then exposed to ambient conditions before being remeasured.

The results demonstrated that most stoppers resealed rapidly after puncture, with minimal change in headspace oxygen levels. This indicated that the stoppers were able to regain closure integrity shortly after needle removal.

In one isolated case, however, a sample exhibited a significant increase in headspace oxygen after multiple punctures, indicating the presence of a permanent leak (Figure 2). As discussed later in this article, this leak was also readily detected by a standard CCI test method using non-destructive headspace carbon dioxide analysis.

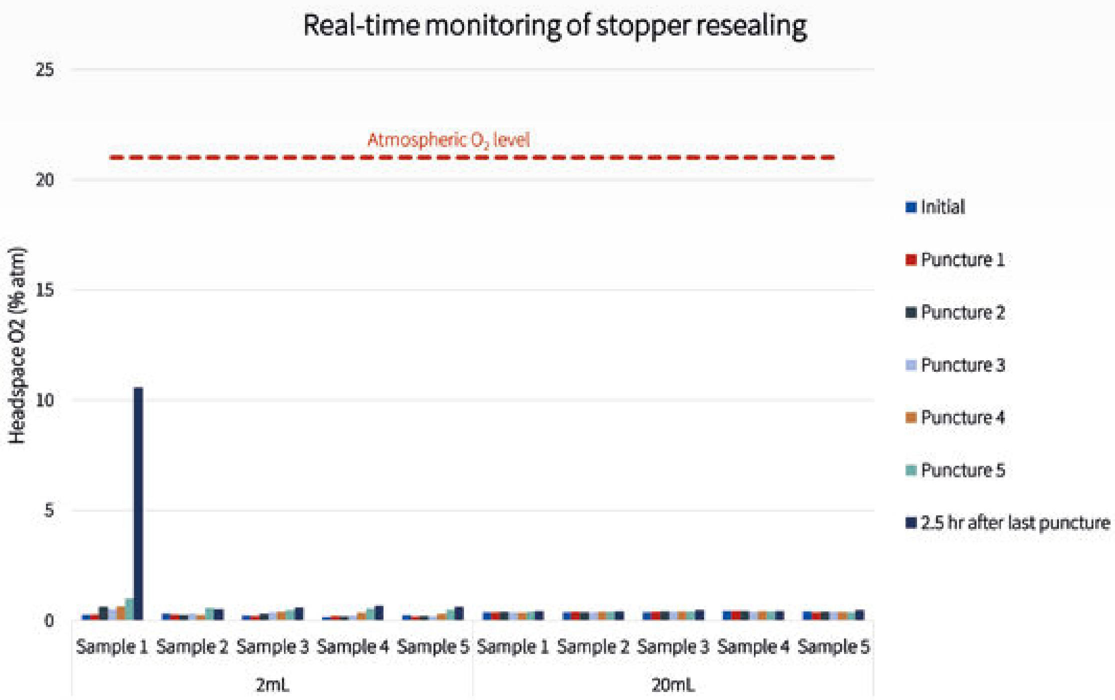


Figure 2 — 2mL and 20mL vials were stoppered and sealed under a nitrogen atmosphere. The vials were then punctured multiple times, and headspace oxygen measurements were made directly after each puncture.

TEST SAMPLES WITH A VACUUM HEADSPACE

These samples mimic a pharmaceutical manufacturing process that may be used for a freeze-dried product. Similarly to the samples with a nitrogen headspace, initial measurements confirmed that the samples were prepared with a low-pressure headspace. During the puncturing protocol, the headspace pressure was continuously monitored to obtain real-time insight into the resealing behavior of the stoppers.

The measurements with the 20mL samples showed that the temporary leak path created by needle removal typically closed within seconds (Figure 3), confirming the self-sealing properties of the elastomeric stopper. Resealing of the stopper was also further confirmed by a standard CCI test method using non-destructive headspace carbon dioxide analysis, as shown later.

It should be noted that the 2mL vial configuration was measured in this manner as well. It was found that due to the relatively small headspace volume, atmospheric pressure levels were already reached after 2 punctures. Resealing performance after the second puncture could thus not be monitored in real-time for this vial configuration.

The next step in the study was to challenge all punctured test samples for CCI, as required by USP <382> section 5.2. Although real-time monitoring of resealing was not feasible for the 2mL vial, this did not impact the ability of our standard CCI test method, using headspace carbon dioxide analysis, to evaluate CCI of the punctured 2mL samples.

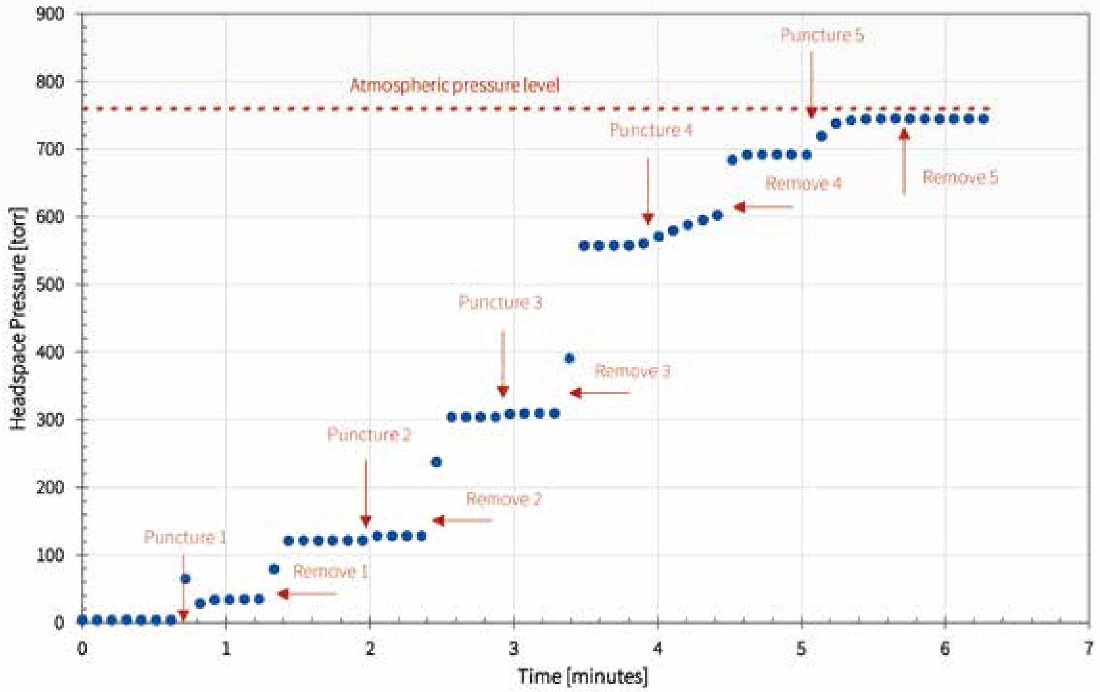


Figure 3 — Continuous headspace pressure measurements of a 20 mL vial being punctured multiple times with a syringe needle. The Vial was prepared with a vacuum headspace, so any increase in pressure is indicative of a leak. If the pressure remains constant, the leak has sealed.

Container Closure Integrity (CCI) Testing Using Headspace Gas Ingress Analysis

In the headspace gas ingress approach, a CCI test vessel is used that can be pressurized with a tracer gas, typically carbon dioxide. Samples are placed inside the vessel and exposed to this controlled carbon dioxide environment during a conditioning phase. If a defect is present in the container closure system that generates a breach in CCI, the tracer gas (e.g., carbon dioxide) will enter the vial through the leak path.

Following conditioning, each container is measured for carbon dioxide levels using highly sensitive, laser-based headspace analysis technology. Containers exhibiting elevated carbon dioxide levels relative to baseline measurements are then identified as having a leak.

Conceptually, this approach is similar to the blue dye ingress test historically used under USP <381>. Rather than relying on subjective visual detection of liquid ingress, headspace analysis leverages the superior sensitivity of laser-based absorption spectroscopy.

Combined with an effusive gas flow conditioning environment, this enables the development of a fully deterministic method that can be validated to reliably detect leak rates critical to maintaining product quality. The result is a more sensitive, reproducible, and non-destructive approach to container closure integrity testing, capable of identifying even the smallest leaks.

Figure 4 illustrates the principle of the method, showing pressurized CO_2 headspace gas entering defective vials in the CCI test vessel during the sample conditioning cycle.

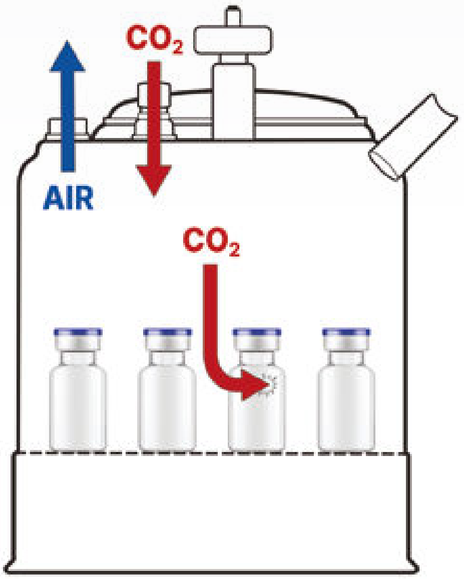


Figure 4 — Schematic representation of conditioning vials inside a carbon dioxide rich environment. After the conditioning period, the vials are measured non-destructively for headspace carbon dioxide levels on a LIGHTHOUSE headspace analyzer.

Developing a headspace gas ingress method following USP <382> and USP <1207>

Following the stopper puncture experiments, all control and test samples were evaluated for container closure integrity with non-destructive headspace gas analysis using carbon dioxide as a tracer gas.

After conditioning, the headspace carbon dioxide content of each vial was measured using a laser-based headspace analyzer. Because the samples originally did not contain a significant amount of carbon dioxide, any measurable increase in carbon dioxide served as a clear indicator of a leak path.

Measurements were taken at three timepoints:

- Before conditioning
- Immediately after removal from the conditioning vessel
- After three hours at ambient conditions

This time window allowed evaluation of potential gas exchange processes while still ensuring reliable detection of leaks.

Gross positive control

Micron-sized positive control

CONTROL SAMPLE PERFORMANCE

Control samples were used to verify the sensitivity and specificity of the method. Baseline measurements confirmed that all samples contained negligible carbon dioxide in their headspace prior to conditioning. After exposure to the carbon dioxide environment, the unmodified controls showed no measurable increase, demonstrating that the intact vials maintained container closure integrity throughout the test (Figure 5).

In contrast, both types of positive controls were clearly identified:

- Gross defects produced immediate and significant carbon dioxide ingress.
- Micron-sized laser-drilled defects were also consistently detected.

The study also showed that detected leaks remained measurable for at least three hours after removal from the conditioning vessel, providing a practical measurement window for routine testing.

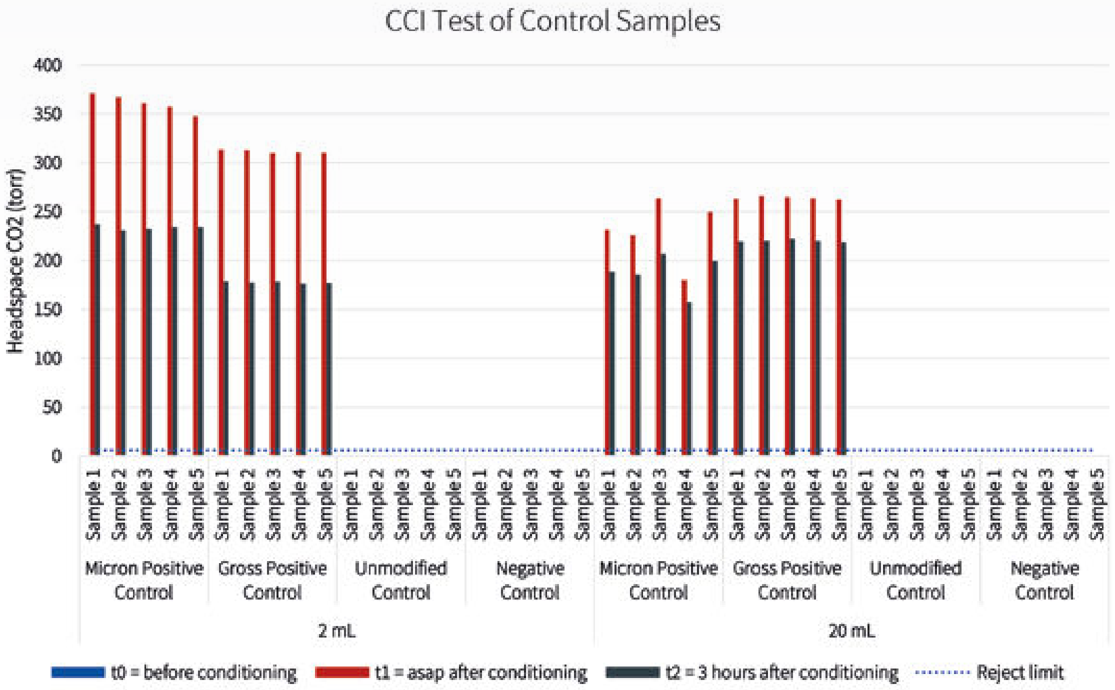


Figure 5 — CCI test results of the control samples that were used to determine method performance. The control samples were all prepared with an air headspace having negligible levels of carbon dioxide. Because carbon dioxide is used as a tracer gas, any measurable increase is indicative of a leak.

TEST SAMPLE RESULTS

For the 20mL vial configuration, all test samples maintained container closure integrity following the puncture protocol.

For the 2mL vial configuration, all samples except one maintained closure integrity during testing. The single failing sample corresponded to the same vial that previously showed elevated oxygen levels after repeated punctures (Figure 2), providing consistent confirmation of a permanent leak.

This finding demonstrates how headspace analysis can easily identify self-sealing failures following needle puncture, providing valuable assurance for multi-dose packaging systems.

METHOD SENSITIVITY AND PERFORMANCE

To define a reliable pass/fail threshold, the measurement uncertainty and baseline carbon dioxide levels were evaluated. Based on these factors, a CCI acceptance criterion of 6 torr CO_2 was established for this study.

Using this criterion, the study confirmed that the headspace gas ingress method can:

- Reliably detect defects with a flow-effective size at least as small as 5 µm
- Maintain high specificity, with no false positives observed in intact samples
- Provide repeatable results across different vial sizes

The gas ingress process can be described by gas flow physics and can predict that the theoretical detection limit of the CCIT method will extend to defects with a flow-effective size down to the sub-micron range, depending on conditioning times and headspace volumes. [1] [2] [3]

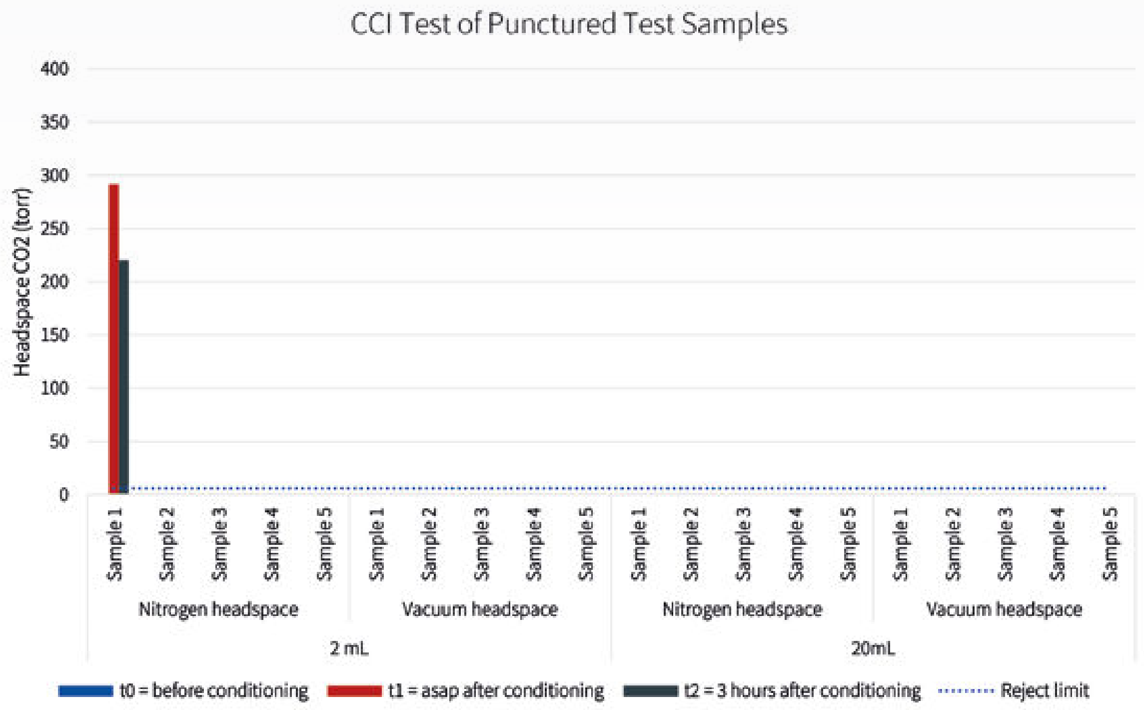


Figure 6 — CCI results of the 2mL and 20mL punctured test samples. Because carbon dioxide is used as a tracer gas, any measurable increase is indicative of a leak.

Advantages of headspace analysis for CCI testing

Beyond its **sensitivity**[4], this study highlights several practical advantages of headspace gas analysis for container closure integrity testing.

First, the method is **non-destructive**, allowing the same sample to be measured repeatedly. This adds usability beyond testing in accordance with USP <382>. The same technology and method approach can be used further down the product life-cycle, such as during stability studies and investigative testing.

Second, the method can identify **both permanent and temporary leaks**. If a container briefly loses integrity under certain conditions, any resulting change in headspace gas composition can still be detected later. This also proves particularly useful for freeze-dried products, where raised stoppers prior to capping can give rise to temporary leaks that cannot be detected with any of the other deterministic methods listed in USP <1207>.

Finally, headspace analysis enables **efficient testing of large sample sets**, making it well suited for container closure system validation and routine quality control applications.

Conclusion

This study demonstrates that headspace gas analysis provides a robust deterministic approach for evaluating container closure integrity and stopper resealing performance.

Using a carbon dioxide tracer gas ingress method combined with laser-based headspace measurements, the developed approach successfully identified defects in both 2mL and 20mL crimp-sealed vial configurations.

The results show that the method can:

- Be validated to readily detect defects with a flow-effective size of at least 5 µm
- Identify leaks caused by syringe needle punctures
- Distinguish defective containers from intact packaging systems.

Together, these results confirm that headspace gas ingress testing offers a powerful and practical solution for evaluating stopper self-sealing performance and container closure integrity in multi-dose vial systems.

Are you ready for USP <382>?

If you need to validate the self-sealing capacity, inherent integrity, or headspace preservation of your parenteral packaging, headspace analysis is the most precise tool for the job. Contact our team of experts today for your specific container-closure system.

REFERENCES

[1] **Method Development for CCI Evaluation via Headspace Gas Ingress by Using Frequency Modulation Spectroscopy** K. Victor, L. Levac, M. Timmins, et al. PDA J Pharm Sci Tech **2017** 71 429-453

[2] USP <1207.1> Section 4.2.4 Detection Limit: "...the limit of detection can be mathematically predicted on the basis of gas flow kinetics and is a function of the time lapse between analyses, and the smallest gas content or pressure change that can be reliably detected by the instrument for the given package system."

[3] USP <1207.2> Section 2.2.2 Application: "...Mathematical models appropriate to leak flow dynamics may be used to predict the time required for detecting leaks of various sizes or rates..."

[4] **Comparing CCI Test Methods - Performance of Headspace Carbon Dioxide Analysis versus Helium Leakage Using Positive Controls** C. Proff, K. Victor, A. Alix Caudill, et al. PDA J Pharm Sci Tech **2024**, 78, 681-698.

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