WHITE PAPER

Container Closure Integrity Evaluation of Molded Vials Stored at -80°C During Stability Using Laser-Based Headspace Analysis

By Lighthouse Instruments and SGD Pharma

The retention of good container closure integrity (CCI) throughout the lifecycle of a pharmaceutical product is crucial. It guarantees that critical medicines remain effective and that the patient stays safe. From numerous studies it is known however, that storage of pharmaceutics at deep-cold conditions significantly increases the risk of compromising CCI. Moreover, the CCI failures that occur, at deep-cold conditions, are mostly temporary, and cannot be detected with traditional methods such as blue dye testing. Laser-based headspace analysis *can* detect these temporary defects, and when combined with robust seal quality testing, suitable packaging components and processing conditions can be selected based on robust scientific data.

While extensive CCI data exists for tubing vials at low temperatures, molded vials have been studied less despite their advantages in mechanical strength, chemical durability, and suitability for sensitive formulations. The study described in this whitepaper, tested 10 mL and 100 mL molded vials paired with two different stoppers and crimped at three compression levels. Samples were stored at –80 °C in a CO_2-rich environment for up to six months. Laser-based headspace CO_2 analysis served as the non-destructive leak indicator, and residual seal force (RSF) measurements characterized sealing quality. Across all vial–stopper combinations and compression levels, no leak was detected in the test samples during storage. The results indicate that molded vials, like tubing vials, provide robust CCI under deep-cold conditions. In addition, it is demonstrated that combining analytical seal quality testing with laser-based headspace analysis supports robust data generation for component selection, process validation, and CCI control strategies.

Introduction

Good container closure integrity (CCI) of primary packaging is crucial in maintaining the stability, sterility, and efficacy of parenteral drug substances throughout their shelf life. Both USP <1207> [1] and Annex 1 to the European GMP guidelines [2] emphasize the importance of using CCI testing (CCIT) and generation of robust data sets to make scientifically sound decisions on both package and process parameters.

New drugs, such as advanced biologics or cell and gene therapies, require storage at very low temperatures to preserve nucleic acid integrity, making seal consistency crucial for pharmaceutical companies. Studies of products stored at -80 °C show that at these temperatures there is a high risk for issues with seal integrity. [3] This is largely due to the physical properties of butyl stoppers which typically have a glass-transition temperature between -55 and -70 °C. At temperatures below this range, the stoppers lose their elasticity. In addition, all components that make up the seal (glass of the vial, rubber of the stopper and the metal crimp seal) shrink at different rates. The loss of stopper elasticity combined with the mismatched shrinkage rates can lead to gaps in the seal, allowing cold, dense non-sterile gases (such as air, nitrogen or carbon dioxide) to enter the container.

The loss in seal integrity is only temporary, as removal from cold storage will make the components re-expand and the stopper regains elasticity, and the seal re-forms—trapping leaked gases inside. Such temporary leaks can compromise product stability and sterility but go undetected by traditional and other deterministic CCI methods. Previous studies have demonstrated that the risk of losing CCI at these low temperatures can be mitigated by a thorough understanding of the packaging components design and the process of their assembly. [3] [4] [5]. One key aspect of reducing the risk of losing CCI lies in controlling the compression force of the stopper.

Currently, a substantial amount of data exists on container closure integrity (CCI) for tubing vials, allowing for a comprehensive evaluation of their sealing performance at low temperatures [3] [6] [7]. In contrast, CCI data for molded vials—particularly at low-temperature conditions—remains limited. Molded glass combines exceptional mechanical resistance, proven chemical durability, and is the best option for delamination issues, which are essential factors for the stability and safety of pharmaceutical products and the patient. Since molded glass is formed in a single step at high temperature, it has a more homogeneous internal surface, which reduces the propensity for delamination. Molded vials are therefore particularly suitable for sensitive injectable drugs - such as high pH formulations, cytotoxic, biological or lyophilized products, and diagnostic contrast agents - because they offer strong protection against mechanical stress, chemical interactions, and particle generation. Beyond these benefits, the growing complexity of pharmaceutical formulations introduces new challenges related to storage and primary packaging performance under extreme conditions.

In this white paper, we present data on the CCI performance of molded vials stored at -80°C for a period of 6 months, evaluated using laser-based headspace analysis. According to USP <1207>, laser-based headspace analysis is considered a deterministic method for CCI testing [1]. This non-destructive technique measures gas concentrations inside sealed parenteral containers with high precision and uses the presence of a tracer gas, that enters the container through a defect, as the basis for detection [8]. This method can readily differentiate between negative and positive controls with a range of micron-sized defects. Importantly, laser-based headspace analysis is uniquely able to detect temporary leaks that may form when products are stored at very low temperatures.

Method & Materials

The CCI study was performed at atmospheric pressure, on sample sets consisting of 10 mL and 100mL molded vials with ISO 20 neck finish, as supplied by SGD. Each vial sample set was sub-divided into two groups and stoppered with either bromobutyl stopper A or B. The serum stoppers were provided by two different suppliers. All vials were then capped and crimped at different stopper compression levels (Table 1). Three different stopper compression settings were used to cover a range of loosely to tightly capped and crimped vials. For CCI testing, controls were manually prepared by Lighthouse using the loose components (Figure 1) by; 1) creating a gross defect by inserting a syringe needle of a capped and crimped vials after removing the cap; 2) creating a micron sized certified defect in the body of the container; 3) sealing a negative control sample, having no artificial defect in the container; 4) sealing an unconditioned negative control samples having no artificial defect in the container. These samples were not exposed to the sample conditioning step.

Table 1: An overview of the sample matrix.

STOPPER COMPRESSION LEVELS	Target Range	STOPPER (SUPPLIER)	NUMBER OF SAMPLES	
			VIAL TYPE: 10 ML	VIAL TYPE: 100 ML
Low	8-10 lfb (36-44N)	A	40	40
		B	40	40
Middle	10-13 lfb (44-58N)	A	40	40
		B	40	40
High	13-16 lfb (58-71N)	A	40	40
		B	40	40

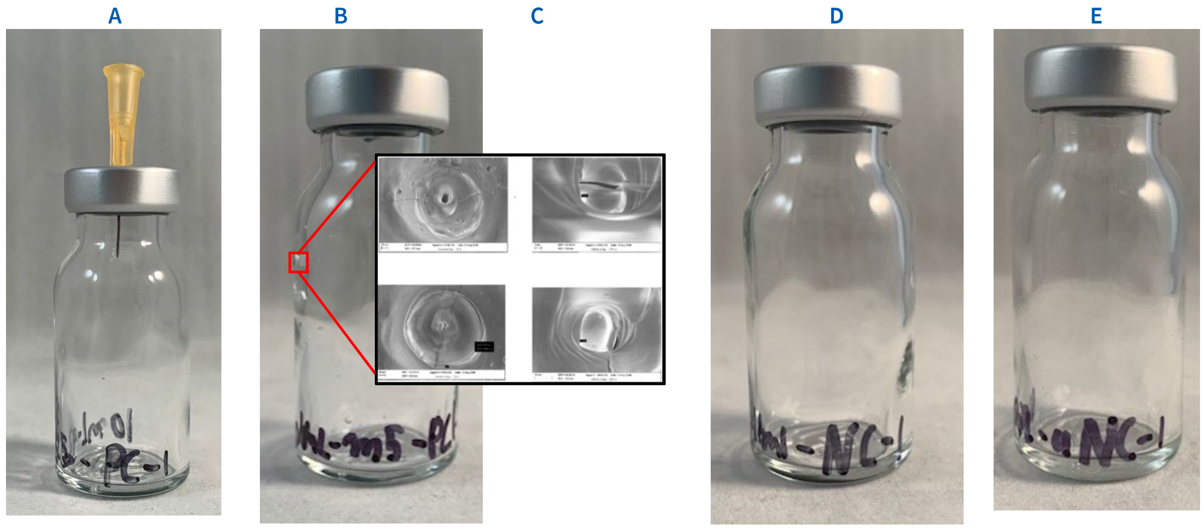

Figure 1: Pictures of empty controls prepared in this study for the 10mL container. Image of A) a gross defect positive control sample with a syringe needle and B) A positive control sample with a micron sized defect and C) zoomed in image as provided by the supplier). D) A negative control.

The samples including positive and the negative controls were placed inside a -80°C freezer containing dry ice. The dry ice created a carbon dioxide (CO_2) rich atmosphere in the storage environment, which means that any leaking containers would ingress CO_2 during storage. An increase of CO_2 measured in a sample after storage indicated a leaking container. If no significant increase in CO_2 levels was measured after the storage period, the packaging was considered to have remained intact. In this scenario, CO_2 served as a tracer gas for leaks and headspace CO_2 measurements using an FMS-Carbon Dioxide Headspace Analyzer were performed directly after deep cold storage served as a CCI indicator which was performed after either 3 months or 6 months of storage. In addition, RSF measurements were performed after the final timepoint using a Residual Seal Force Tester.

Results & Discussion

Both the 10mL and 100mL molded vial configurations, with the two different stoppers, were prepared, stoppered, capped and crimped with a crimping force resulting in different stopper compression levels. The results show that with increasing crimping force, the mean RSF value per set increases. Figure 2 shows the boxplot of the 10mL molded vial configuration as an example of the spread of the RSF values over the different compression levels.

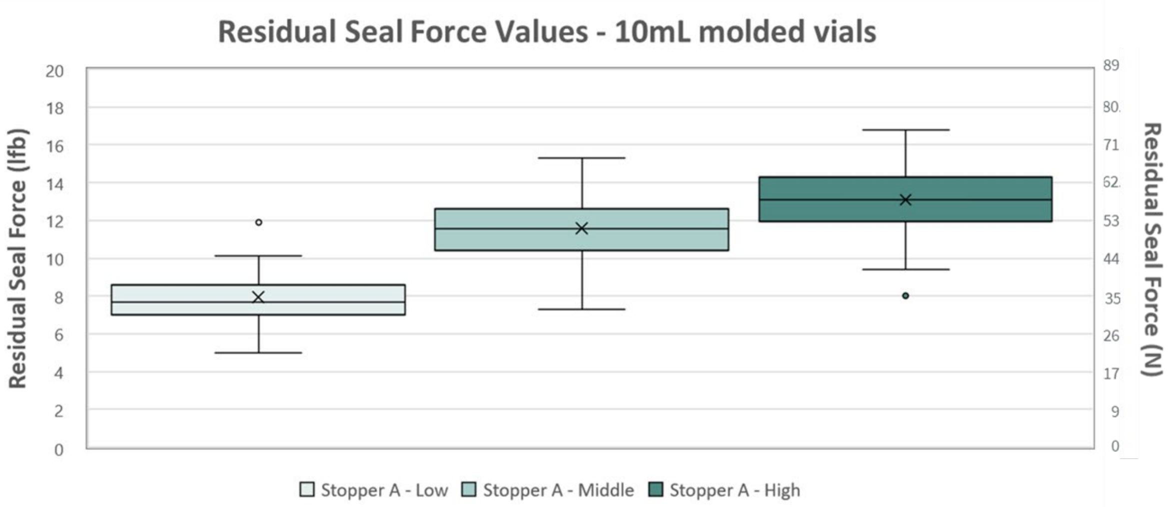

Figure 2. Boxplot of the Residual Seal Force measurements on 10 mL molded vials with stopper A. 40 samples were analyzed for each crimp setting. X = Mean; Horizontal line = Median, Whiskers = Minimum and Maximum value within the 1.5 Interquartile range (IQR)) bounds).

The carbon dioxide limits of detection and quantitation (CO_2-LoD and CO_2-LoQ) were determined according to the ICH Q2 guidelines [8]. The CO_2-LoD for the FMS in combination with the standard set used was estimated as ~0.5 mbar and ~0.4 mbar, while the CO_2-LoQ was estimated to be 1.5 mbar and 1.1 mbar for the 10 mL and 100 mL configurations, respectively.

The negative controls showed no ingress of CO_2 during the storage periods. While the positive controls showed elevated levels of CO_2 during the storage periods as expected (Figure 3). This demonstrates that that the presence of a leak in the samples would be readily detected and distinguished from a non-leaking sample. Table 2 shows that for all tested vial-stopper combinations and stopper compression levels, the samples showed no elevated levels of CO_2 after storage at -80°C ± 10°C in a CO_2 rich environment up to 6 months - indicating maintenance of CCI over this period.

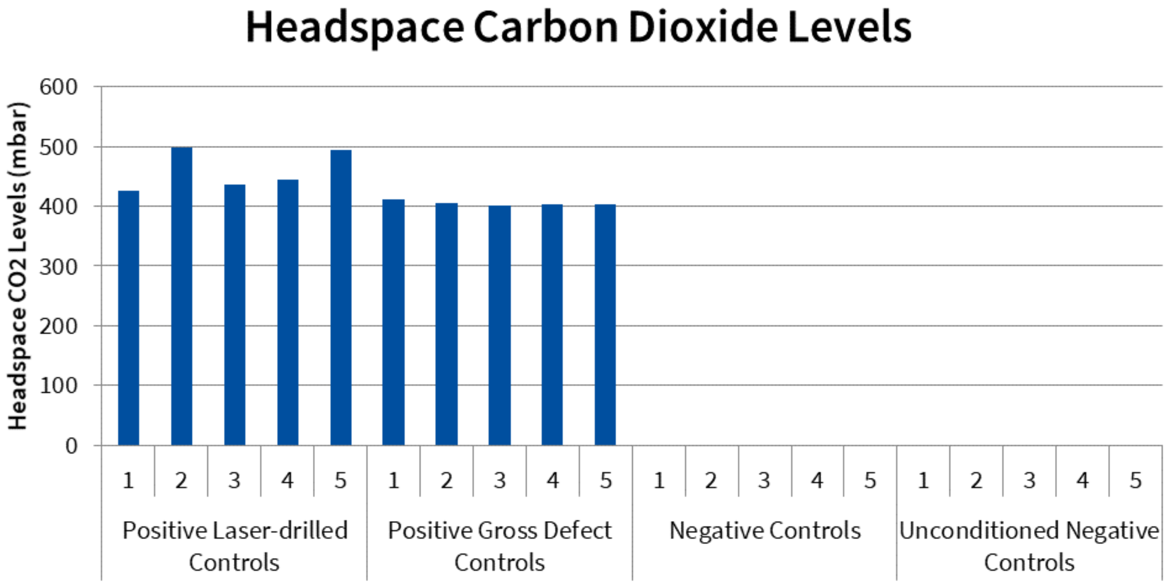

Figure 3: Graph of headspace carbon dioxide levels of the control samples at the 3 months timepoint when measured after the test samples.

Table 2: An overview of the number of samples showing CCI loss indicative by increase CO_2 levels for each vial-stopper combination and stopper compression level.

VIAL TYPE	STOPPER COMPRESSION LEVELS	STOPPER	CCI TESTING TIMEPOINTS	
			T_3 MONTHS	T_6 MONTHS
10 mL/100mL Molded vials	Low	A	0/40	0/40
		B	0/40	0/40
	Middle	A	0/40	0/40
		B	0/40	0/40
	High	A	0/40	0/40
		B	0/40	0/40

Conclusion

- Molded containers show good CCI performance for both stoppers over the full range of stopper compression during storage at -80°C for a period of up to 6 months.

- No elevated levels of CO_2 were observed in the tested samples, while positive controls confirmed the method's ability to detect leaks, demonstrating the sensitivity and reliability of headspace analysis as a CCI test.

- The capability to generate reliable seal quality data through RSF testing over the tested range and CCI data through non-destructive headspace analysis supports multiple stages of the product life cycle. This includes the selection of suitable primary packaging components, validation of capping and crimping processes, and the establishment of a robust CCI control strategy.

References

[1] USP, "Package Integrity Evaluation – Sterile Products," in *United States Pharmacopeia*, U. S. P. Convention, Ed., 2017, p. 1707.

[2] EudraLex, "Annex 1: Manufacture of Sterile Medicinal Products, Sections 8.21-8.25, 8.28," in *The Rules Governing Medicinal Products in the EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use*, vol. 4, 2022, pp. 24-25.

[3] B. Zuleger, D. Duncan, U. Werner, A. Kort, R. Glowienka and E. Wehnes, "Container/Closure Integrity Testing and the Identification of a Suitable Vial/Stopper Combination for Low-Temperature Storage at -80 C," *PDA J. Pharm. Sci. Technol.,* vol. 66, no. 5, p. 453, 2012.

[4] "Lighthouse Instruments eBook: Container Closure Integrity and Deep Cold Storage.," 13 October 2020. [Online]. Available: https://www2.lighthouseinstruments.com/l/302881/2020-10-13/kntqq.

[5] "Lighthouse Instruments Application Note: Deep Cold Storage Effects on CCIT," [Online]. Available: https://www2.lighthouseinstruments.com/l/302881/2017-12-19/wm3k.

[6] M. Edey, J. Wilmer, D. Duncan, A. Rozentsvayg, S. Mulhall and V. Valley, "A holistic science-based approach to container closure integrity for parenteral products: Lessons learned from a vaccine requiring deep cold storage using a conventional vial system," *European Journal of Pharmaceutics and Biopharmaceutics,* vol. 214, no. 114791, 2025.

[7] K. Victor, A. A. Caudill and J. Veale, "Container Closure Integrity Test Method Development on Vials Stored at -80°C Using Headspace Carbon Dioxide Analysis," *PDA J Pharm Sci Technol,* vol. 76(5), pp. 395-415, 2022 Sep-Oct.

[8] A. Caudill, K. Victor, M. Timmins and J. Veale, "Container Closure Integrity Test using Frequency Modulation Spectroscopy Headspace Analysis with Carbon Dioxide as a Tracer Gas," DOI. 10.5731/pdajpst.2020.011502, 2020.