Whitepaper

Saying Goodbye to Blue Dye: How to Navigate The Changing World of CCIT

A comparative study of different **container closure integrity test methods** on **packaging** for **pharmaceutical products**

PFEIFFER VACUUM

Content

1. Introduction

As the first layer of defence against the outside world, the importance of a pharmaceutical product's primary packaging is not to be underestimated. From blister packs to vials, ampoules, syringes and auto-injectors, the goal of all pharma packaging is to protect the drug from outside contaminants, enabling safe storage and transportation to patients. Effective primary packaging also facilitates accurate dosing in many products such as vaccines.

Defects in the packaging can have serious consequences, potentially causing the drug to leak from the container. At the same time, there is the potential for external contaminants, moisture, and oxygen to enter the container, affecting the product's sterility and stability and thus potentially harming the patient or reducing the efficacy of the drug.

While relatively uncommon on a well set-up and maintained packaging line, occasional defects in the primary packaging are inevitable. It's how a company tests, identifies, and measures them that can make the difference.

To ensure a robust packaging system, leak testing is also highly important during the development of new products and in stability studies, which assess the integrity of the packaging across the entire shelf-life of the drug. As the pharmaceutical industry continues to implement innovative new packaging and drug delivery systems with more components and greater complexity, such validation studies become even more critical.

A range of leak testing methodologies have been devised to test container closure integrity (CCI), and each one comes with a unique set of advantages and limitations. In this whitepaper, we explore the current CCI testing (CCIT) landscape and compare probabilistic and deterministic leak testing methods to determine which is fit for the future of pharmaceuticals.

2. The changing regulatory landscape

Regulations govern everything the pharmaceutical industry does, with the overall aim of ensuring the safety and efficacy of approved drugs for patient use. Considering the critical role of primary packaging in this mission, healthcare bodies have naturally stressed the importance of CCIT as a key assay in quality control.

As stated by the US Food and Drug Administration (FDA), "CCI is considered an essential part of suitability, especially in the aspect of protection against microbial contamination, reactive gases (e.g. oxygen) and moisture. A container closure system that permits penetration of microorganisms is unsuitable for a sterile product."

While there are a range of testing methods available, the pharmaceutical industry has relied on a method known as the blue dye ingress test for decades. In recent years, changes to the regulations are urging a move away from this practice.

In 2016, the US Pharmacopeial Convention updated Chapter 1207 of its USP guidance, encouraging a move towards deterministic methods. A similar outlook was echoed by the European Medicines Agency (EMA) in 2022, where updates to its EU GMP Annex 1 stated that final containers closed by fusion should be subject to 100% integrity testing using validated methods, with visual inspection alone "not considered as an acceptable integrity test method". According to the EMA, where containers are closed by methods other than fusion, "a scientifically justified sampling plan should be used."

The revision also announced that containers closed by fusion should be subject to 100% integrity testing, and that samples of other containers should be checked for integrity using validated methods and in accordance with quality risk management.

While probabilistic methods like the blue dye ingress test were considered appropriate 30 years ago, the regulatory bodies would like to see a move to validated, deterministic methods for new drug products. But what exactly do these terms mean for CCIT?

3. Understanding probabilistic and deterministic test methods

As set out in the USP Chapter <1207.1>, a probabilistic method measures a series of sequential and/or simultaneous events, with a random outcome based on probability distribution. Results are subjective and qualitative. Large sample sizes and rigorous test condition controls are required to obtain meaningful results.

Deterministic methods, on the other hand, measure a predictable chain of events. According to the USP, "leakage is measured using physicochemical technologies that are readily controlled and monitored, yielding objective quantitative data. Many deterministic leak test methods rely on the predictable movement of gas that inevitably occurs through an open leak path".

3.1 Probabilistic: the blue dye ingress test

The blue dye ingress test is a probabilistic test. To follow this method, a small number of samples are placed in a vacuum chamber with a blue dye solution. The air is first evacuated and the chamber is returned to atmospheric pressure or a slight overpressure. This pressure differential causes the dye to penetrate through defects in the container, which can then be inspected visually by an operator.

Thanks to its simplicity, convenience, and low cost, this test remains widely used in the industry. However, in addition to the abovementioned limitations of probabilistic methods, the test is operator-dependent, destructive, and low in sensitivity.

More advanced leak testing assays and technologies have since been established to provide deterministic, efficient, and reliable methodologies to the pharmaceutical industry. On the simplest level, these newer methods operate the same way as the blue dye ingress test, simply measuring with gas and sensors what the blue dye test measures with liquid and an operator's eye. This concept has been developed by Pfeiffer Vacuum, who offers three key test methods to replace the blue dye ingress test.

3.2 Deterministic methods and technologies

There is no one-size-fits-all approach to CCIT, and pharma companies are required to perform different tests throughout the lifecycle of a drug, right from the product development stage and stability testing to in-process controls (IPCs) across the manufacturing line.

Pfeiffer Vacuum offers three deterministic and non-destructive CCIT technologies to the pharmaceutical industry: helium mass spectrometry, Mass Extraction technology, and optical emission spectroscopy.

3.2.1 Helium mass spectrometry

Tracer gas leak testing is a popular deterministic method of CCIT. This test requires the presence of tracer gas inside the test container, with helium being the most common tracer gas followed by hydrogen. A vacuum is used to draw gas out of any leak channels and orifices, while a mass spectrometer quantitatively measure the leakage rate of tracer gas from the container.

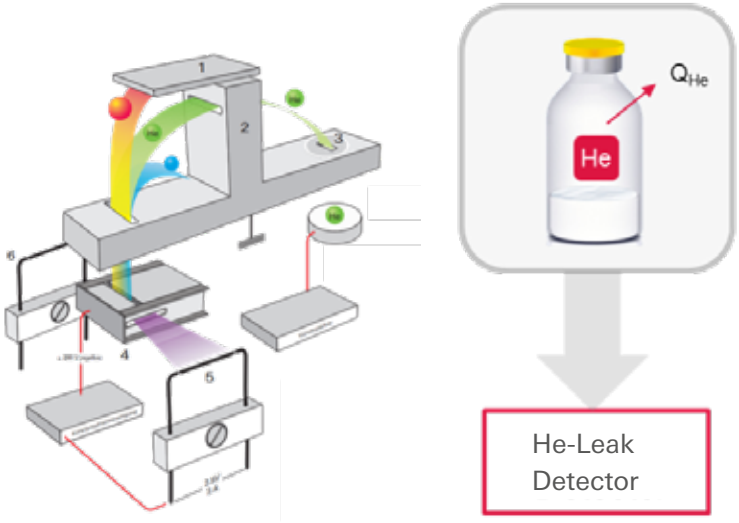

Figure 1: Schematic overview of the operating principle of helium mass spectrometry

Any methods involving the use of tracer gas can be destructive or non-destructive depending on whether the container is open (empty) or closed (filled). For this reason, methods like helium mass spectrometry are typically used in applications involving the initial validation of a new packaging system, rather than IPCs or mass production testing. This is an efficient, high-speed test, and is often considered the gold standard in leak detection sensitivity due to helium's ability to penetrate even the most minute defects. It can be challenging to set up due to the requirements of helium gas management, however.

There are many different products for helium mass spectrometry on the market depending on their purpose. Some of them have multi purposes as well as the one's which concentrate on special features like for example sniffer leak detectors. Also for the CCIT world there are special versions existing which focus on the automated tool handling and filling of the samples as this was found as a major point which influences the final results. Offered by Pfeiffer Vacuum, the ASM 2000 is a comprehensive solution developed for design and validation studies of new packaging systems. An automated charging module first fills the packaging containers with helium. When the vacuum is pulled, any leakage of helium is detected and measured by highly sensitive mass spectrometry analysis.

3.2.2 Mass Extraction technology

In this patented system from Pfeiffer Vacuum, containers are placed inside a test chamber connected to a vacuum reservoir, which is evacuated. Due to the differential pressure, gas escapes through prevailing leaks in the containers and flows in the direction of the vacuum storage tank. The leakage rate of the test unit is determined by the flow from the unit to the vacuum reservoir, which is measured by highly sensitive Micro-Flow technology.

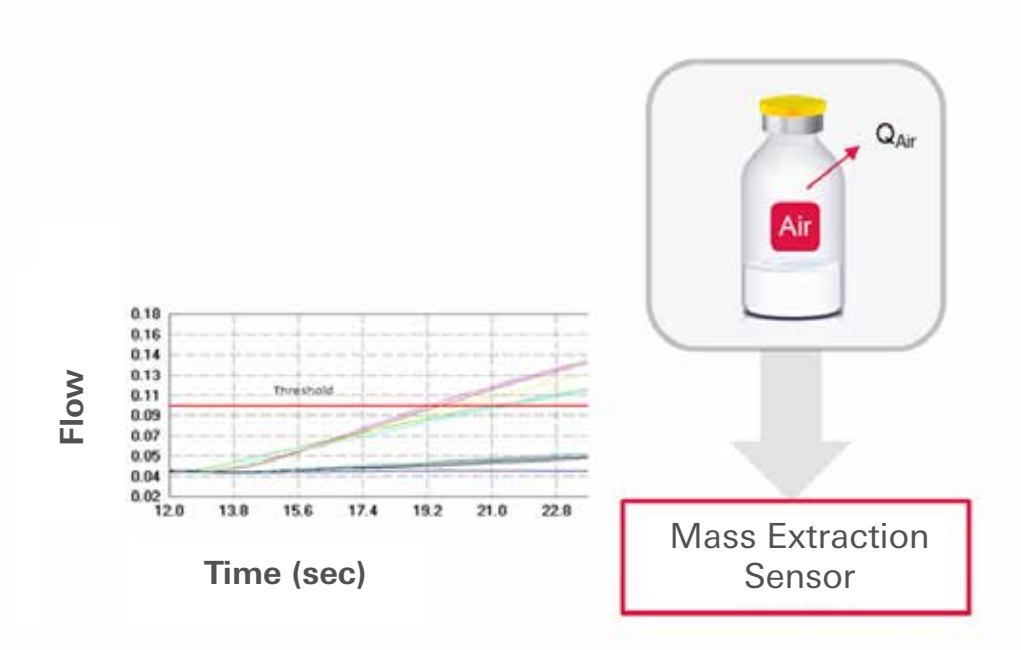

Figure 2: Schematic overview of the operating principle of Mass Extraction

Due to its non-destructive nature, Mass Extraction (usage of Micro-Flow in vacuum) leak testing is well suited to IPCs, where a set percentage of a batch is taken to quality control. It is a robust technology which is easy to set up and possible to automate. In addition, no tracer gas is needed.

The high speed of the test and versatility when testing different drug / packaging systems make Mass Extraction a great option for the pharmaceutical industry. It is worth noting, however, that this method can be sensitive to temperature and/or volume variations.

3.2.3 Optical emission spectroscopy

This innovative technology deploys a multi-gas sensor based on optical emission spectroscopy to track the flow of different gases escaping from a leaky container exposed to vacuum. No specific tracer gas is required as Pfeiffer Vacuum's unique AMI sensor can detect the gas already present in the container, including argon, nitrogen, carbon dioxide or air.

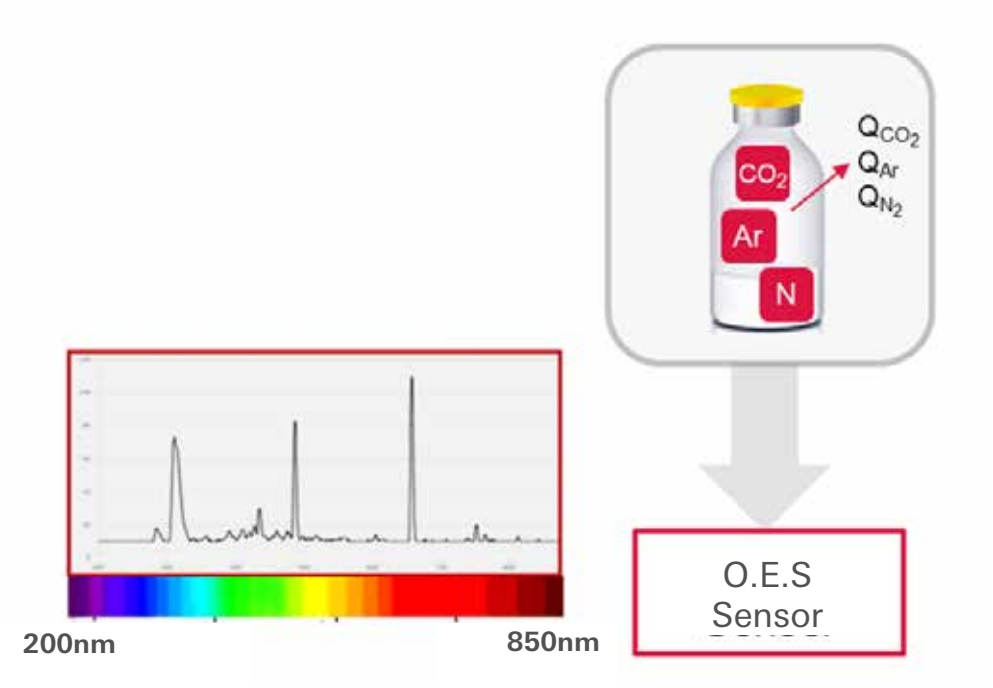

Figure 3: Schematic overview of the operating principle of optical emission spectroscopy

The benefit of optimal emission spectroscopy is its non-destructiveness, sensitivity, convenience, and high speed. As with all CCIT technologies developed by Pfeiffer Vacuum, the test is highly deterministic, objective, and comes with software that is traceable as per the FDA's 21 CFR Part 11 standards.

The technology can test multiple containers with high sensitivity at the same time, although the detection limit depends on the packaging and drug type as well as the gas used for detection. In addition, the test chamber can be customised according to the needs of the product dimensions and the quantity of products tested simultaneously.

4. A scientific review

4.1 Understanding the maximum size of a tolerable leak

While there are many factors to consider when selecting a CCIT method, including cost, speed and destructiveness, the most important factor is often sensitivity – can the method be trusted to detect miniscule defects, such as those in the range of 1–10 µm and even lower?

In the late 90s and early 00s, several scientific studies were conducted to understand the maximum size of a tolerable leak to preserve sterility. The first landmark study was published by Kirsch et al in the PDA Journal of Pharmaceutical Science & Technology, in 1997. Kirsch studied the correlation between helium leak rates and microbial ingress in rubber-stoppered glass vials subjected to test units immersed in a 35 degrees C bath containing magnesium ion and 8 to 10 logs of viable P. diminuta and E. coli for 24 hours. Micropipette defects were used, and the study sought to determine at what leak size no microbial ingress was observed, verified with helium mass spectrometry.

At large leak rates, the probability of microbial ingress approached 100% while at very low leak rates, microbial ingress rates were 0%. Kirsch determined that a helium leak rate of approximately $6 \cdot 10^{-6}$ std cc/sec correlated with a <10% probability of microbial ingress. Below a defect size of approximately 0.2 µm the probability of microbial ingress is below 0% according to Kirsch's study.

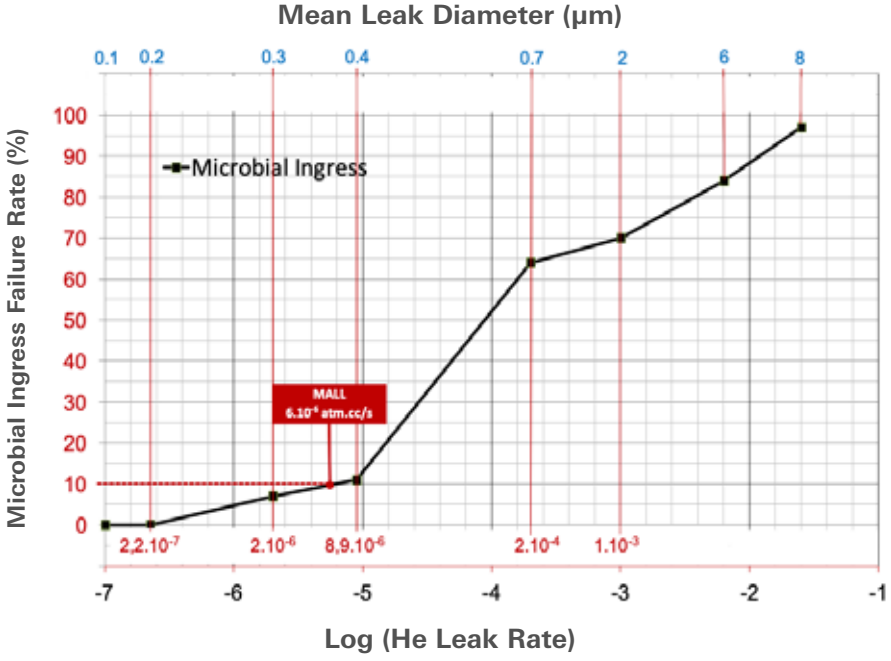

Figure 4: Microbial ingress failure rate versus leak size in µm and mbar*l/s [1]

This finding formed the basis of an important CCIT concept known as the maximum allowable leakage limit (MALL). This limit is defined in USP Chapter <1207> as "the greatest leakage rate (or leak size) tolerable for a given product packaging/delivery system that poses no risk to product safety and has no impact, or inconsequential impact, on product quality."

Another study was published in 2000 by Burrell et al, who investigated the correlation of dye ingress and microbial ingress. In this study, 3 cm-long capillary defects were used and it was shown that both methods had a similar leak detection rate. However, these tests had an approximately 70% chance of detecting a 10 µm leak, and any leaks below 5 µm were non-detectable. Considering Kirsch's MALL was in the range of 0.2 µm, this demonstrates that it is not enough to talk about a defect size, but that the type of defect also plays an important role when evaluating proper leak testing methods.

4.2 A scientific comparison of the blue dye test and deterministic methods

In addition to the development of high-quality, advanced leak detection systems, Pfeiffer Vacuum is also at the forefront of research in the CCIT field. The company recently conducted a study comparing the sensitivity of the blue dye ingress test with its three technologies: helium mass spectrometry, optical emission spectroscopy (OES), and Mass Extraction (ME).

Method

All tests were performed on 20 ml glass vials. Mirroring the studies of Kirsch et al and Burrell at el, two types of defects were used:

- 3 cm-long capillaries
- Glass micropipettes

Vials were drilled on their side at 30 mm from the bottom to allow the placement of the defect through their wall.

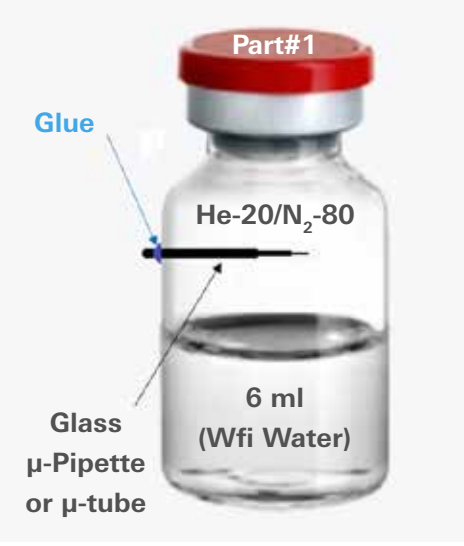

Figure 5: Preparation of vial to be tested

To assess the detection limits of each CCIT method, a range of diameters for each defect were glued and tested. 30 vials were tested for each diameter (total 420), meanwhile 60 additional vials were glued without defects and used as negative controls throughout the study.

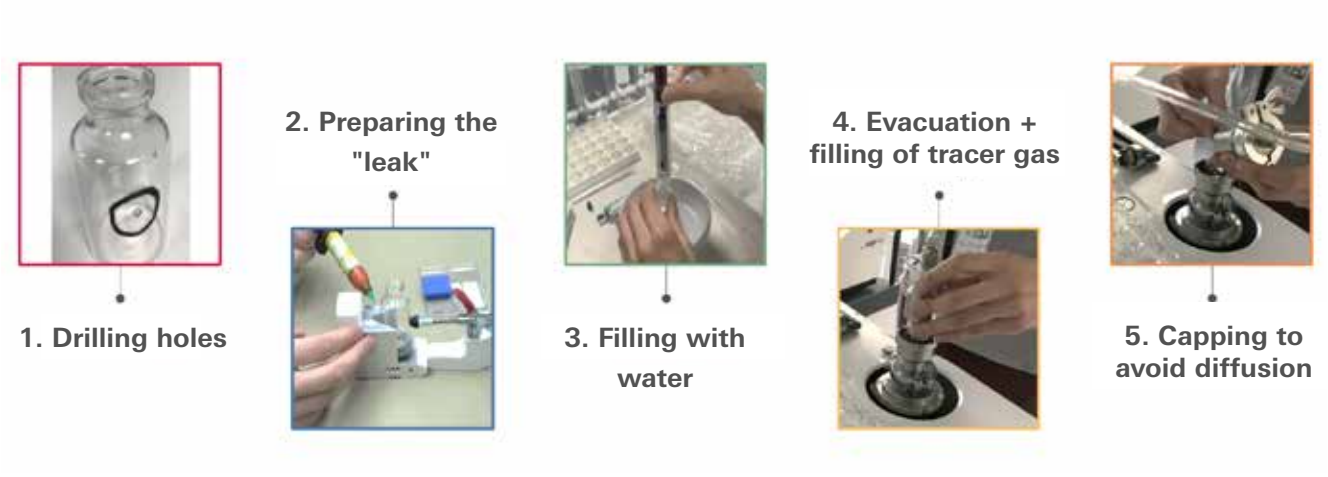

Figure 6: Process of preparation for execution of comparative study

All vials were filled with 6 ml of Water For Injection (WFI) and a gaseous mix of 20 % helium and 80 % nitrogen, then caped and directly measured with helium right before to avoid diffusion through the rubber stopper occurs. The defects were positioned above the water line. Figure 6 shows the step-by-step process for the preparation of the vials.

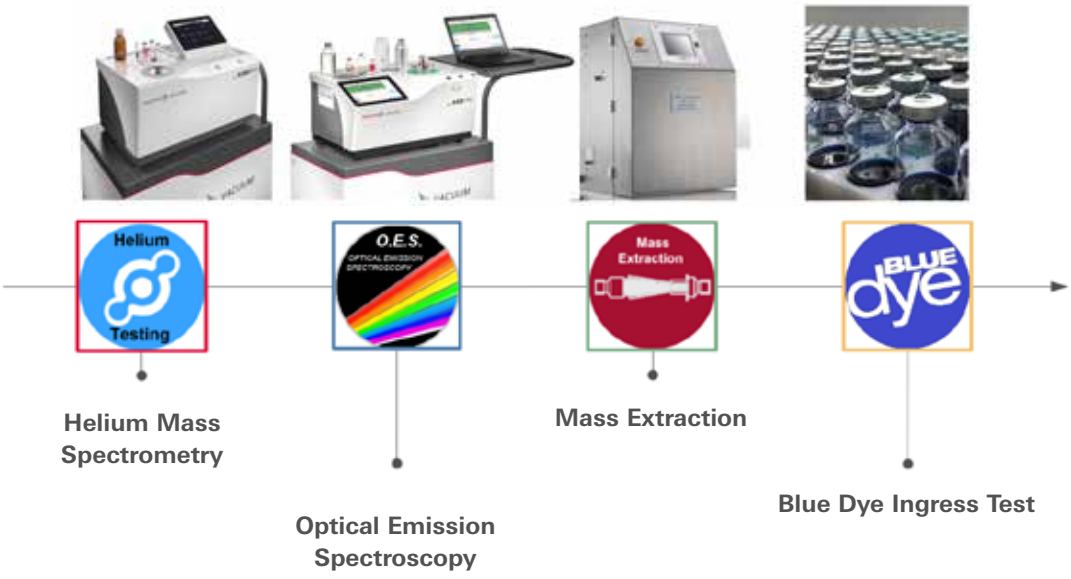

Figure 7: Order of the test procedures performed

As shown in Figure 7, samples were first tested with helium, followed by OES and ME, before the final test with blue dye. The device used for helium mass spectrometry throughout this study is an ASM 2000 from Pfeiffer Vacuum. For OES, Pfeiffer Vacuum's AMI 1000 system was used, and for ME the company's ME2 was used. The recipes were set based on the usual testing parameters used with Pfeiffer Vacuum's devices.

The blue dye recipe was set to ensure detection of 100% 20 µm * 3 cm capillaries upon visual inspection. Test conditions were based on the modified ISO guidelines and consisted in 60 minutes at –37 kPa followed by 30 minutes at atmospheric pressure. Vials were manually cleaned and then inspected by three different operators, who compared them to control vials. If at least two out of three readings were positive for a vial, it was then considered leaky.

Figure 7.1: Vial with capillary (left) and with micropipette (right) after blue dye test

Results

Corresponding averages and standard deviations were assessed for both the leaky and tight samples. For a defect type to be considered detected, its average minus three times its standard deviation (sigma) would have to be superior to the blanks' average plus three times their standard deviation (sigma) as illustrated in figure 8.

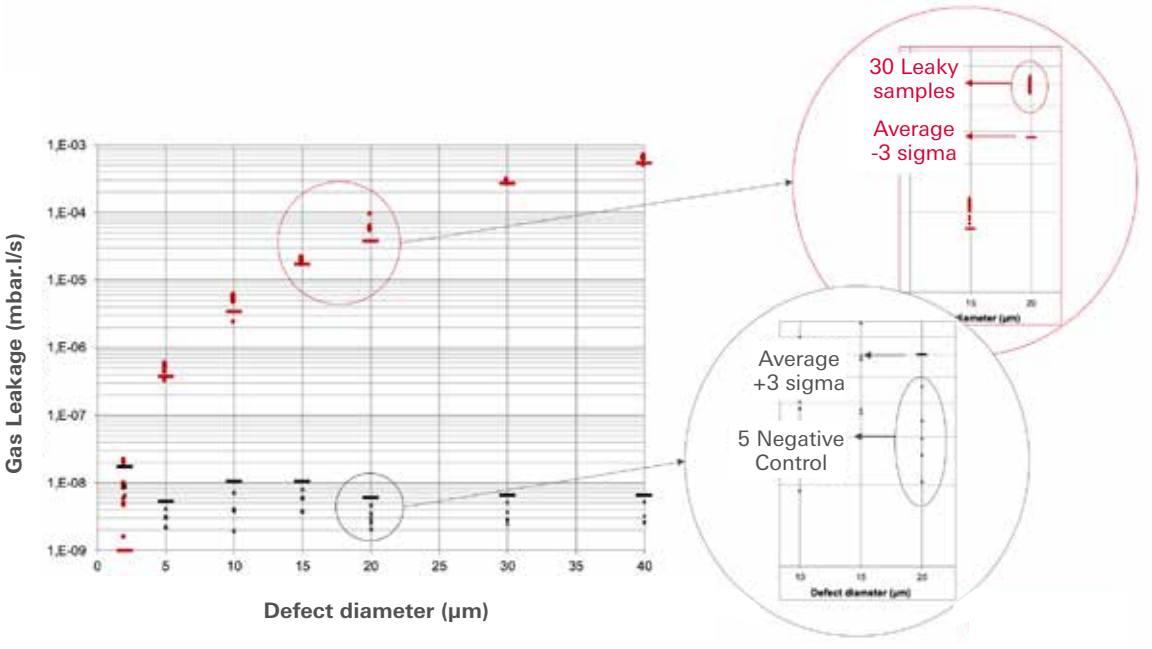

Figure 8: Definition of pass fail criteria by standard deviation (3 sigma)

The graphs below show the measured detection limits of each technology for micropipettes and capillaries.

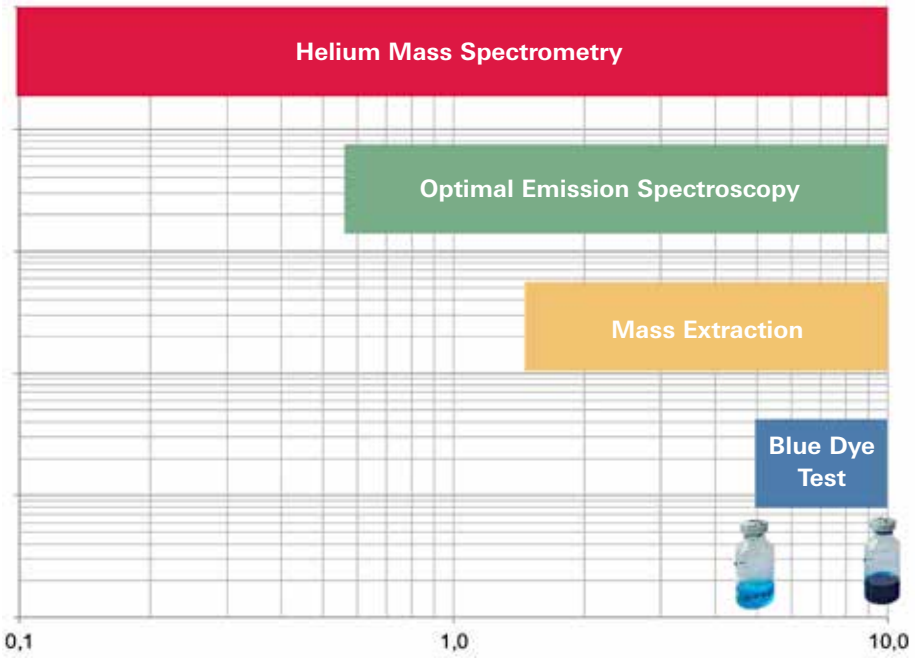

Figure 9: Detection limits of glass micropipettes for each method

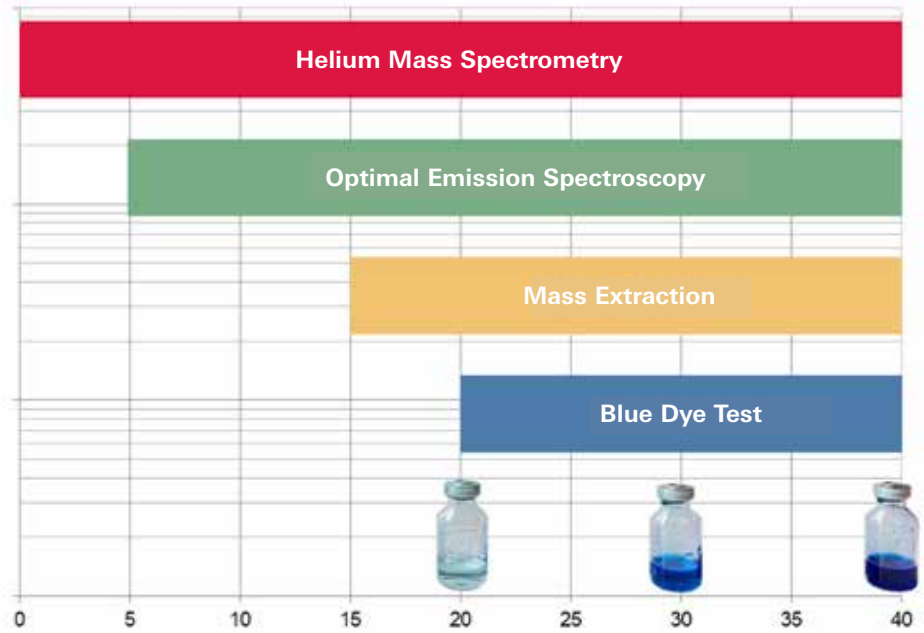

Figure 10: Detection limits of capillaries for each method

With a detection limit of 5 µm for micropipettes and 20 µm for capillaries, the blue dye ingress test shows the lowest sensitivity. Helium mass spectrometry proved to be the most sensitive method across both defect types, with detection limits of 0.1 µm for micropipettes and 4 µm for capillaries, though defects below < 4 µm could be detected with the use of a higher helium concentration.

Showing detection limits of 0.6 µm for micropipettes and 7 µm for capillaries, OES was the second most sensitive test after helium mass spectrometry. This was followed by ME with 1–2 µm for micropipettes and 15 µm for capillaries.

Additional points

While Pfeiffer Vacuum followed an immersion time of 60 minutes for the blue dye test, test conditions and immersion times vary between companies. In the relevant ISO guidelines, an immersion time of 30 minutes is recommended. However, on a recent poll conducted by Pfeiffer Vacuum, 33% of respondents stated they use an immersion time between ten and 30 minutes, and 32% selected less than ten minutes. This suggests that the detection limits for the average blue dye ingress test performed by the pharmaceutical industry would be much higher than the results of this study. The blue dye test is also highly depending on the size of the package tested. It was necessary to double the immersion time to reach the same sensitivity mainly due to the bigger headspace in the sample.

Meanwhile, the full capabilities of helium mass spectrometry were not explored in this study. While the detection limit of helium for capillary defects was recorded as 4 µm in the study, it should be noted that filling the vials with a greater percentage of helium would significantly improve the results, enabling the detection of capillaries as small as 1–2 µm or less.

Conclusion

This study confirmed some important information for the pharmaceutical industry, namely that the blue dye ingress test has a significantly lower sensitivity level than the deterministic methods. Of the three deterministic methods tested, helium mass spectrometry displayed the most sensitivity, even when testing containers with helium concentration of only 20%. By using 100% He, defects in the range of <0.1 micron for glass capillaries and <4 micron capillaries are also measurable.

Cycle time is another important parameter when evaluating CCIT methods. For the blue dye ingress test, this depends on the batch size. Cycle time is often in the range of ten minutes, though longer immersion times are recommended. On the other hand, leak tests with helium mass spectrometry and OES can be performed in 25 seconds or less. The cycle time for this very sensitive measurements with Mass Extraction is approximately 75 seconds. Bigger defects can be detected faster.

Moreover, the three deterministic methods by Pfeiffer Vacuum provide quantitative, objective results compared to the qualitative, subjective results of the blue dye ingress test. This means the pharmaceutical company does not only get the information of a leak present, but will get valuable information about the size of the leak. As a result, the industry can use the measurement data for post-treatment analysis.

5. Navigation to deterministic CCIT

5.1 Reasons and advantages of deterministic methods

It is clear from Pfeiffer Vacuum's study that deterministic methods such as helium mass spectrometry, ME, and OES provide stronger results than the blue dye ingress test. Yet while the regulatory authorities would like to see the industry expand its use of these technologies, there is currently no rule to say that the pharma industry *must* switch. As such, many companies may not see the value in changing to a more costly test method until it becomes mandatory.

Other companies, however, are realising the strategic value of investing now. The increased regulatory requirements for higher sample sizes in IPCs, for example, is putting pressure on quality control departments. Operators desire a fast system with high throughput that enables them to do their job more efficiently, hence why many companies are cutting corners with the immersion time of the blue dye ingress test.

As the industry trends towards high-value biologic products manufactured in low volumes, the financial consequences of a faulty packaging system are becoming more and more severe.

Helium mass spectrometry can be performed in as little as 25 seconds. Both Mass Extraction and optical emission spectroscopy can be automated and are an ideal way to bring IPCs up to date with the rest of the manufacturing line, as factories transform operations in accordance with the Industry 4.0 vision.

Quality control is becoming increasingly important for pharma companies, too. As the industry trends towards high-value biologic products manufactured in low volumes, the financial consequences of a faulty packaging system are becoming more and more severe. With some drugs, the cost of losing just one dose would surpass the price of investing in an advanced leak testing system.

5.2 Tips for moving from blue dye ingress test

It can be daunting to change a system your company has relied on for years, but there are many reasons why now is the right time to switch. Here are the top three things you should keep in mind when making your decision.

1. Evaluate the sensitivity level of your blue dye test

Most of the time, the detection limit of the blue dye ingress test in practice has not been verified. If you are considering changing your CCIT method, the best place to start is with an assessment of your current system and its sensitivity. Then, it is important to assess whether this is appropriate for your product and test approach.

To understand the full capabilities and limitations of your current system, pharma companies may send test samples to an external company or leak testing solutions provider, who will help them evaluate their method by comparing it to others on the market.

Pfeiffer Vacuum offers test method validation studies from its application laboratories in Annecy, France, and Indianapolis, US. By sending your test samples to one of these two locations, Pfeiffer Vacuum will conduct a small study to provide you with a deterministic test result using its CCIT methods. Test samples are sent back to you, enabling you to determine the sensitivity of your dye ingress test and understand when it's time to upgrade to a deterministic CCIT method.

2. Understand the requirements of your packaging system

When choosing between CCIT methods, it's important to consider its compatibility with your complete packaging system, meaning the combination of the packaging configuration and the product formulation itself. Keep in mind any limitations of the system, such as clogging effects with large protein drugs (which may block leak artefacts) or that the blue dye test is relying on a bunch of parameters that affect the test result: i.e. bigger head space. At the end choose a method that fits best with your product over one that is preferable overall.

3. Define your desired test results

Your end reason for conducting the test will also have a big impact on the type of system you end up deploying. This often depends on which manufacturing stage you plan to use the test, and whether 100 % testing is required or not. For example, while helium leak detection is considered the gold standard in sensitivity, it is not always feasible to fill samples with tracer gas. For the validation of a new packaging configuration, however, this may be the best technology.

6. Conclusion

6.1 Comparision of different CCIT methods

CCIT is an essential part of the packaging process, and its importance is growing in the eyes of the regulatory bodies. In this era of modern manufacturing and Industry 4.0, it no longer makes sense to use a CCIT method that relies on an operator's opinion, is time consuming, and lacks traceability. Most importantly, deterministic methods such as those offered by Pfeiffer Vacuum are known to improve the sensitivity, capability and reliability of your integrity test.

The recent scientific study conducted by Pfeiffer Vacuum has proved one of the principal limitations of the blue dye ingress test: its high detection limits (lack of sensitivity). Meanwhile, the results were much stronger for the deterministic methods, particularly for helium. Helium mass spectrometry is frequently considered the most sensitive technology on the market for this reason.

However, as discussed, there are many pros and cons that must be weighed up for each method, and the decision of which system to use depends on a wide variety of factors. Here is a comparison table summarising some of the key points that must be considered.

	Blue Dye Ingress Test	Helium Mass Spectrometry	Mass Extraction	Optimal Emission Spectroscopy
Deterministic	--	+	+	++
Non-destructive	-	0	+	+
Objective	-	+	+	+
Tracebale	-	+	+	+
Sensitive	-	++	0	+
Easy to set-up	++	--	++	++
High speed test	-	+	0	++
Automated	--	--	+	+

Figure 11: Comparison of different test methods

6.2 Key takeaways

Evaluate the sensitivity level of your blue dye test
- Is it appropriate for your product and test approach?
- Compare results against deterministic test methods
- Pfeiffer Vacuum offers test method validation studies

Understand the requirements of your packaging system
- Consider the CCIT method's compatibility with your packaging and drug
- Keep in mind any limitations such as clogging
- Always choose the method that best suits the product

Define your desired test results
- At what manufacturing stage will you perform the test?
- Do you require 100 % testing or random?
- Decide your test approach, then choose the appropriate method for the specific application

If you need support, get in touch with our leak test experts from Pfeiffer Vacuum.

leak-testing-services@pfeiffer-vacuum.de

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