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Science and Technology Feature

A proposed scientific rationale and methodology for the establishment of acceptance criteria for leak rates in pharmaceutical freeze dryers

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A scientific rationale is proposed for the establishment of acceptance criteria for leak rates in pharmaceutical freeze dryers. A methodology has been developed to determine the theoretical quantity of air that could leak into a pharmaceutical freeze dryer and, based on the potential maximum bioburden and particulate level of the leaked air from potentially unclassified areas, calculations can be undertaken to determine the maximum allowable leak rate that will maintain Class 100/Grade A conditions for the duration of the lyophilisation cycle. It is shown that the 2 x 10^{-2} mbar-litre/s specification, that is frequently quoted as the acceptable leak rate for modern pharmaceutical freeze dryers, is not appropriate for freeze dryers of differing volumes with lyophilisation cycles of differing lengths.

Introduction

Summary

Assurance of the vacuum integrity of freeze dryers used for the manufacture of sterile pharmaceutical products is essential for GMP operations; however, there is currently no generally accepted scientific rationale for establishing acceptance criteria for such testing[1].

As it stands, current acceptance criteria are generally based on equipment capability either proposed by the manufacturer, or from data collected during qualification of a new freeze dryer. The targeted specification is often one cited by The Parenteral Society, "A frequently specified leak rate for new, clean, dry, and empty freeze-dryers would be 2 x 10^{-2} mbar-litre/s. The leak rate should not change significantly during the life of the freeze-dryer ..."[2].

It should be noted that the 2 x 10^{-2} mbar-litre/s specification only refers to the process capability of freeze dryers and does not ensure or confirm sterility of the manufactured product. The sterility of the manufactured product will be dependent on not only leak rate but also the air quality outside of the freeze dryer, the volume of the internal chamber of the freeze dryer and the time the product remains under vacuum within the freeze dryer. All these elements should be considered when a qualitative risk assessment for leak rate is considered in order to ensure that the maximum leak rate limit is appropriate for the equipment and cycle being used.

Hardwick *et al*.[3] sought to define a scientific justification and methodology of acceptance criteria for freeze dryer leak rate testing, which was published as "A proposed rationale and test methodology for establishment of acceptance criteria for vacuum integrity testing of pharmaceutical freeze dryers". In this assessment, calculations based on the Ideal Gas Law were performed to arrive at the maximum allowable pressure rise (leak rate) value that would maintain Class 100/Grade A conditions for each dryer using worst-case potential bioburden for leaked air.

Discussion

Class 100 (US Food and Drug Administration (FDA)) and Grade A (European Union (EU)) specifications concerning active microbial counts are similar – an action level of one colony forming unit (CFU) for each cubic metre of air space for Class 100[4] and an average limit of <1 CFU/m^3, with "appropriate alert and action limits" for Grade A[5]. Likewise, cleanrooms and clean air devices should be classified in

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accordance with EN ISO 14644-1 which defines Grade A air as having a maximum permitted number of particles per m^3 as 3520 x 0.5 µm and 20 x 5 µm both at rest and in operation. The FDA states, "Facility design should ensure that the area between a filling line and the freeze dryer provide for Class 100…"[3]; however, it does not make reference to requirements for the inside of the freeze dryer. EU guidance is clearer: "Partially stoppered freeze-drying vials should be maintained under Grade A conditions at all times until the stopper is fully inserted"[4].

It may be argued that process control is demonstrated with media fills, but regulatory guidance is sparse, and sometimes seemingly contradictory. The FDA states, "Media fill studies should closely simulate aseptic manufacturing operations…For lyophilization operations, FDA recommends that unsealed containers be exposed to partial evacuation of the chamber in a manner that simulates the process. Vials should not be frozen and ensure that the medium remains in an aerobic state..."[4]. Although these recommendations simulate the air turbulence associated with freeze-drying, while still maintaining the viability of the growth medium, they create conditions that do not simulate the total freeze dryer cycle, per the FDA definition: "Lyophilization – Drying in which the water vapor sublimes from the product after freezing"[6].

Current guidance of leak rate testing for freeze dryers

EU media fill guidelines contain the following recommendation for all media fills (although nothing specific for lyophilised products), "The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical subsequent manufacturing steps"[5].

For vacuum integrity (leak rate) testing of freeze dryers, no official limits are dictated per the FDA. A general discussion states, "Leakage into a freeze dryer may originate from…the atmosphere into the vessel itself...It is necessary to monitor the leak rate periodically to maintain the integrity of the system…Should the leak rate exceed specified limits, determine the actual leak site for purposes of repair. Thus, it would be beneficial to perform a leak test at some time after sterilization…The time and frequency…will vary and will depend on the data developed during the cycle validation. The pressure rise found acceptable at validation should be used…during production."[7]

EU guidance makes one leak test reference (pertaining to moist heat sterilisers), "There should be frequent leak tests on the chamber when a vacuum phase is part of the cycle." The only indication that specifications should be established is this guidance, "The efficacy of any new procedure should be validated, and...verified at scheduled intervals based on performance history…"[5].

Methods

Leak rate test

A typical leak rate cycle will be determined by[8]:

$$\text{Leak rate} = \frac{(\textit{Finish pressure} - \textit{start pressure})\ x\ \textit{volume}}{\textit{Elapsed time}}$$

The pressure of the leak rate test is typically undertaken at the expected working pressure of the vessel (typically between 100 to 10 µbar); for this analysis, a vacuum level of 10 µbar will be used as "worst-case" conditions. Typically, leak rate tests run for at least 1 hour.

Calculation of hole size per given leak rate at 10 µbar (1 Pa)

The Ideal Gas Law is[9]:

$$PV = nRT$$

where P is the absolute pressure (SI unit pascals), V is the volume of gas (SI unit cubic metres), n is the amount of gas (SI unit moles) and T is the thermodynamic temperature (SI unit kelvin). The gas constant R is expressed in the same physical units as molar entropy and molar heat capacity 8.314 $J \cdot mol^{-1} \cdot K^{-1}$

then: $n = PV/RT$

The area of a hole may be derived from kinetic theory[10] where vm, the mean velocity of gas can be expressed as:

$$v_m = \sqrt{\frac{8RT}{\pi M}}$$

where w is defined as the mass of molecules striking per area to give a pressure (from the gas law) and M is a mol of gas (for air = 0.029 kg mol^{-1}).

$$P = \frac{4w}{vm\ M}$$

From a differential form (leak rate) of the gas law the area of a hole may be defined as:

$$\text{Area of hole} = R_L \frac{\sqrt{2\,\pi\,RTM}}{PRT}$$

For SI units, a leak rate RL of 0.02 mbar L/s is 0.002 Pa m^3s^{-1}, (1 Pascal = 0.01 mbar), room temperature is 298K and atmospheric pressure is $1.013x10^5$ Pa.

$$\therefore\ \text{Area} = 1.69 \times 10^{-10}\ m^2$$

or a diameter of 14.68 μm.

Estimate of velocity for a 0.02 mbar L/s leak rate

$$\text{Velocity of leak} = \sqrt{2\Delta P/p}$$

Where ΔP is pressure differential and p is density of the fluid. Density of air is 1.225 kg/m^3

Pressure differential from atmospheric (101,300 Pa) to chamber at 10 µbar (1 Pa)
= 101,299 Pa

Velocity of leak = 406 ms^{-1} however velocity of gas will be limited to speed of sound at 330 ms^{-1} as a maximum flow speed through the leakage spot and this value will be used for the analysis.

Volume flow for a leak rate of 0.02 mbar L/s

$$\text{Volume flow rate} = \text{Area of hole} \times \text{velocity } ms^{-1}$$

However, the actual flow profile downstream of the hole is quite complex, and the volumetric flow rate for real flows typically include a flow coefficient[11] C_f. The flow coefficient is found from experimentation and ranges from 0.6 to 0.9 for most orifices as it depends on the hole shape and length of transit (as well as the Reynolds

Table 1. Estimated leak rate in litres per hour given a leak rate in mbar L/s.

Leak rate mbar L/s	Hole size m^2	Leak flow rate $m^3 s^{-1}$	Estimate leak rate (litres per hour)
0.001	8.46E-12	2.78E-09	0.01
0.005	4.23E-11	1.40E-08	0.04
0.01	8.46E-11	2.79E-08	0.08
0.015	1.27E-10	4.19E-08	0.12
0.02	1.69E-10	5.59E-08	0.16
0.025	2.12E-10	6.98E-08	0.20
0.03	2.54E-10	8.38E-08	0.24
0.035	2.96E-10	9.78E-08	0.28
0.04	3.39E-10	1.12E-07	0.32
0.045	3.81E-10	1.26E-07	0.36
0.05	4.23E-10	1.40E-07	0.40
0.06	5.08E-10	1.68E-07	0.48
0.07	5.92E-10	1.96E-07	0.56
0.08	6.77E-10	2.23E-07	0.64
0.09	7.62E-10	2.51E-07	0.72
0.1	8.46E-10	2.79E-07	0.80
0.15	1.27E-09	4.19E-07	1.21
0.2	1.69E-09	5.59E-07	1.61
0.25	2.12E-09	6.98E-07	2.01
0.3	2.54E-09	8.38E-07	2.41
0.35	2.96E-09	9.78E-07	2.82
0.4	3.39E-09	1.12E-06	3.22
0.5	4.23E-09	1.40E-06	4.02

Number and surface roughness within the hole). As no assumption is made for this analysis on the actual hole size or shape (or multiple holes), a typical value of 0.8 will be used for C_f.

Volume flow rate = Flow coefficient x area of hole x velocity ms^{-1}

Leak flow rate = 4.47 E-08 m^3s^{-1}

Or 0.16 L per hour

From this calculation, **Table 1** was developed relating leak rate in mbar L/s to estimate leak rate in litres per hour.

Determination of potential external contamination rates for assessment

Non-viable particles (NVP)

To determine the "typical" external air quality for the risk assessment, air was sampled outside the freeze dryers within the Ipsen Biopharm manufacturing facility in Wrexham to be used for the analysis. A total of 39 samples of 1 m^3 air were sampled using a Climet independent air sampler (ID 143389) between 30 November and 7 December 2018. This was during a lyophilisation cycle and included engineering activity in the plant room area, so "worst-case" conditions would have been sampled.

Data summary

A summary of 5 µm and 0.5 µm particle results are shown in **Figures 1** and **2**, respectively. The data analysis NVP are given in **Table 2**.

As the maximum particle numbers were generated during engineering work, this would be considered to be the "worst-case" potential for NVP contamination typically seen in the unclassified areas outside the freeze dryers; for 0.5µm, the maximum value of 5150 was obtained which was equivalent to the mean plus 3.8 standard deviations, and for 5 µm the maximum value of 220,220 was obtained which was equivalent to the mean plus 4 standard deviations. These values will be used for the assessment giving adequate process capability for the analysis.

Table 2. Results of data analysis NVP.

Size	Mean	Standard deviation	Maximum
5 µm particle	793	1143	5150
0.5 µm particle	72,854	36,485	220,220

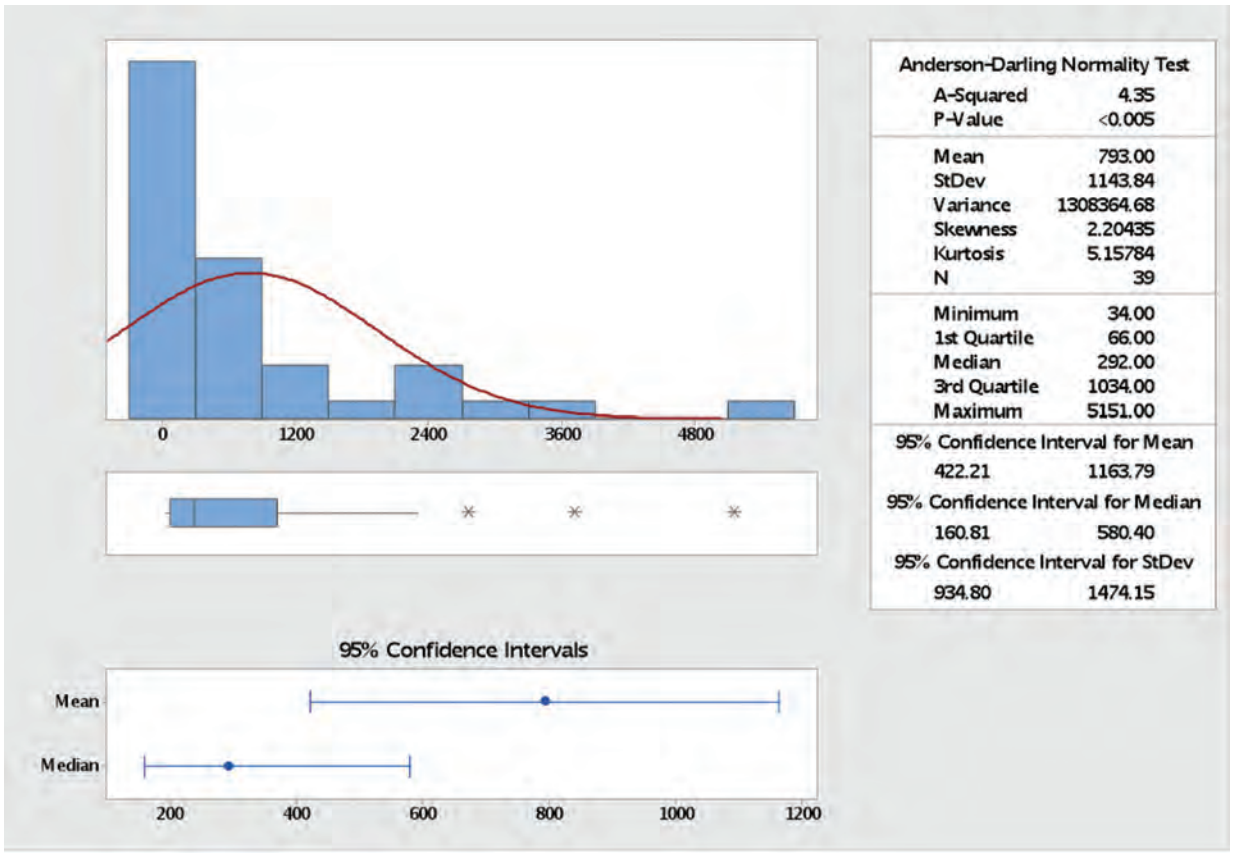

Figure 1. Summary of 5 µm particle results.

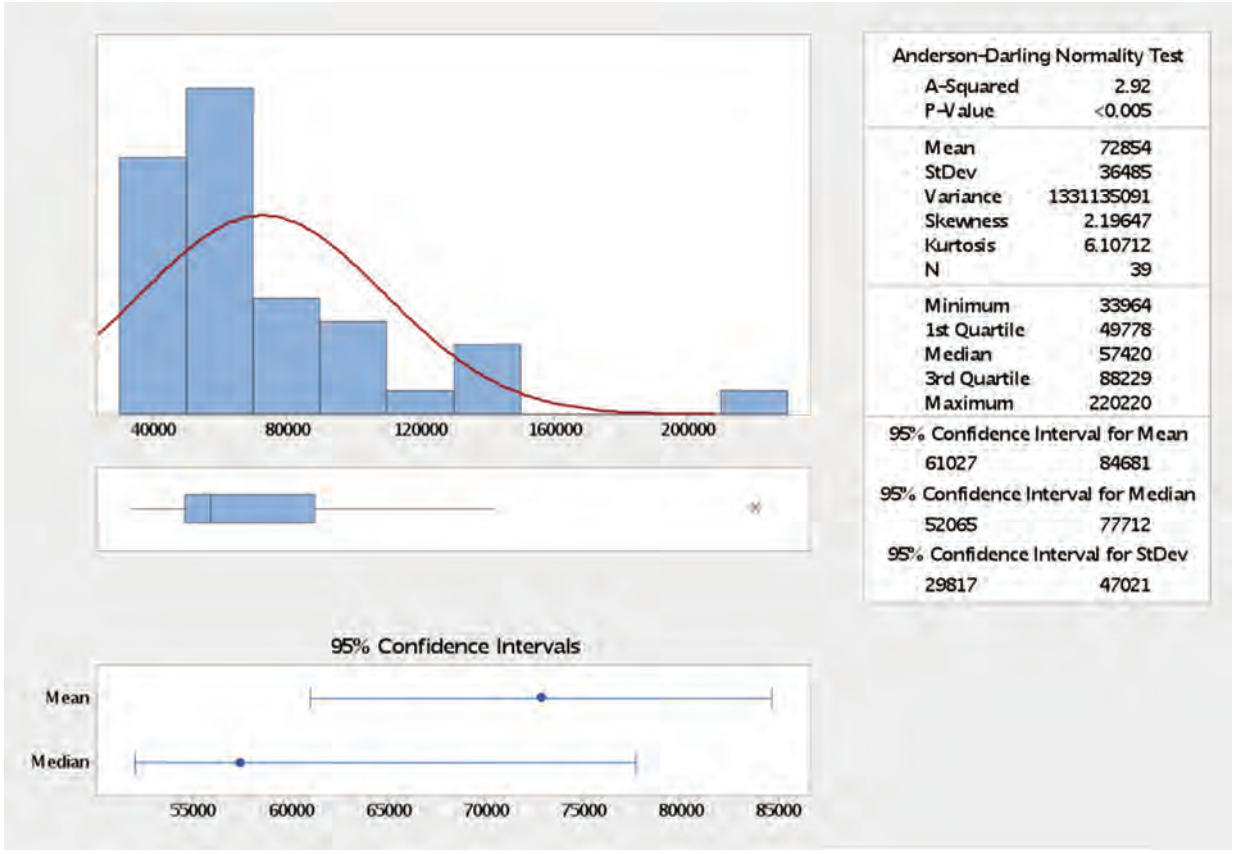

Figure 2. Summary of 0.5 µm particle results.

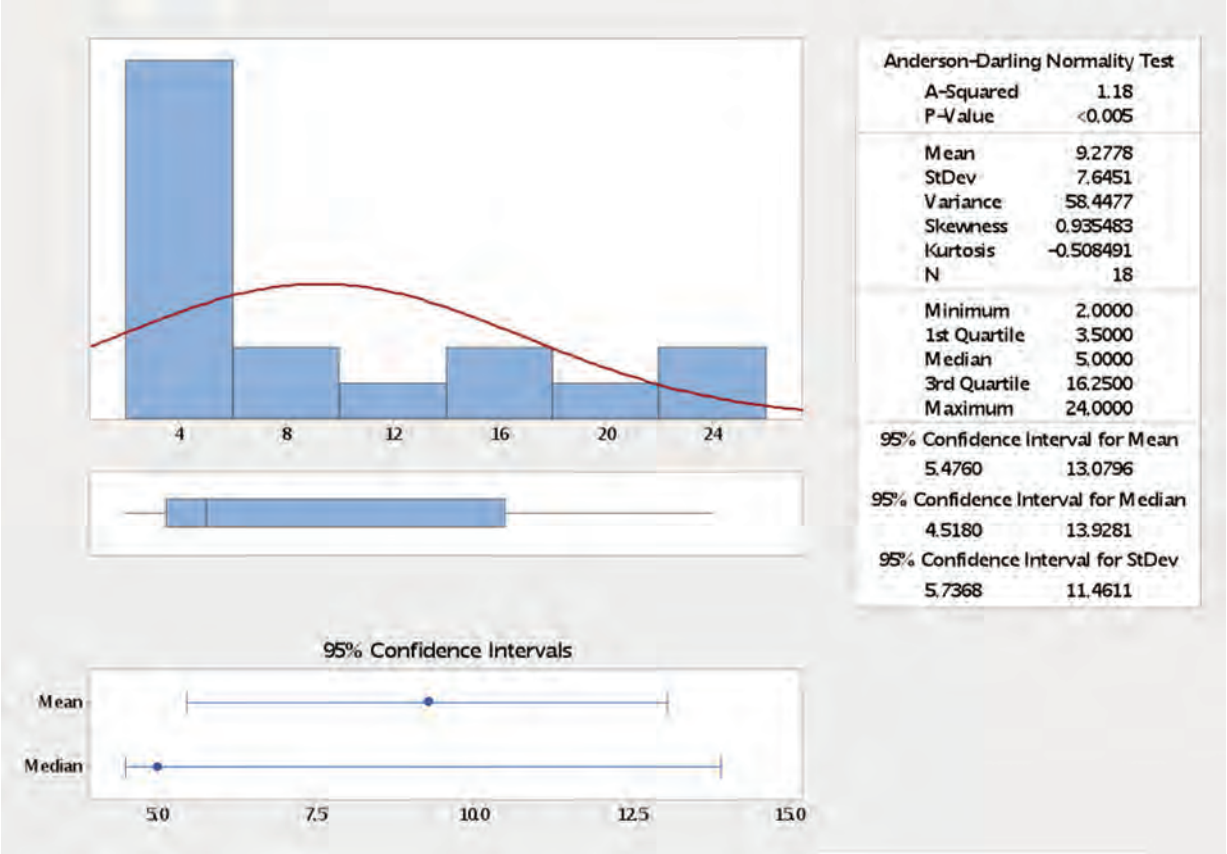

Figure 3. Summary of CFU/m^3.

Bioburden

To determine the "typical" external air quality for the risk assessment, air was sampled outside the freeze dryers within the Ipsen Biopharm manufacturing facility in Wrexham to be used for the analysis. A total of 18 samples of 1 m^3 air were sampled using a MAS-NT active air sampler between 5 and 7 December 2018. This was undertaken during a lyophilisation cycle and included engineering activity in the plant room area, so "worst-case" conditions would have been sampled.

Data summary

A summary of CFU/m^3 is shown in **Figure 3**. The data analysis for CFU are given in **Table 3**. As the maximum CFU numbers were generated during engineering work, this would be considered to be the "worst-case" potential for bioburden contamination typically seen in the unclassified areas outside the freeze dryers; the maximum value of 24 was obtained. This data supports the "worst-case" conditions previously identified in Hardwick *et al*.[3] of 300 CFU/m^3; at 12 times the maximum number obtained, these values will be used for the assessment giving adequate "worst-case" conditions for the analysis.

Assumptions for assessment

For this assessment the following assumptions were made.

1. Although leaks can occur in numerous places in a freeze-drying system, to create a worst-case scenario and to allow for mathematical calculation, the leak is considered to be from a source(s) large enough to admit microorganisms, and located in a part of the system housed in an unclassified mechanical room (with no set specifications and no routine monitoring performed). For this analysis, no recognition is made that the leak will likely consist of multiple pathways and that the probability of a particle entering the chamber is extremely low due to the effect of the length and route of the leakage path leading to direct interception, inertial impaction and charge effects of the particle.
2. Risk to the product from a system leak is limited to the time from the end of primary drying to stoppering of the vials (i.e. all of secondary drying). During primary drying, product risk is minimal because the vigorous rate of mass transfer of water vapour from the product out through the open slot in the product-stopper during sublimation creates a positive pressure in the vial headspace. Matter that might enter the system from the outside will likely travel to the area of lowest pressure, the ice on the condenser, and stay there. Since it

Table 3. Results of bioburden (CFU/m^3).

Mean	Standard deviation	Maximum
9	89	24

is difficult to determine when exactly positive headspace pressure ceases, duration of risk is defined as the entire time the product dwells in the chamber from the end of primary drying to stoppering of the vials.

3. The leak rate remains approximately constant over the time course of secondary drying.
4. The bioburden for the non-classified areas air is 300 CFU/m^3as previously defined[3].
5. The NVP particle level for the non-classified areas air in particles per m^3 is 220,220 x 0.5 µm and 5150 x 5 µm both at rest and in operation.
6. There is no influence of temperature and pressure on this analysis, Hardwick *et al*.[3] matrixed hypothetical results, using the range of room temperatures measured on the days of testing (16–27°C), and using the extremes of barometric pressure that were recorded (979–1043 mbar) which established that varying the temperature did not change the calculated values for the maximum allowable leak flow rate with a higher barometric room pressure allowing for higher passing pressure rise results.
7. It will be assumed that any bacteria entering the chamber will survive a significant pressure differential, transitioning at the speed of sound from atmospheric pressure to 10 µbar.
8. No assumption will be made on the flow or leaks being directed to the point of lowest pressure for the chamber, which will be the vacuum pump, and any particles would follow the expected path of least resistance (dynamic pumping) and be expelled from the chamber. It will be assumed that any material entering the chamber will remain in the chamber as a worst-case assumption, being deposited on the internal chamber.
9. The internal fittings inside the freeze dryer will not be considered and internal volume will be assumed by engineering specification.

Sterility assurance for lyophilisation

EU guidance states: "Partially stoppered freeze drying vials should be maintained under Grade A conditions at all times until the stopper is fully inserted"[4]. Grade A air is defined as <1 CFU/m^3, therefore to ensure that the freeze dryer remains at Grade A conditions, the maximum hold time of product within the freeze dryer can be calculated based on the freeze dryer volume.

Assuming external air quality of 300 CFU/m^3 (or 0.3 CFU/L)[3] and NVP particle levels of 220,220 x 0.5 µm and 5150 x 5 µm per m^3, outside the freeze dryer that can be drawn into the chamber, a calculation can be undertaken of the maximum time that a leak rate can occur before the established action level of Grade A air is exceeded (i.e. one CFU for each cubic metre of air space would be reached and an NVP level of 3250 x 0.5 µm and 20 x 5 µm for each cubic metre of air space was exceeded).

For a leak rate of 0.02 mbar L/s, it has been shown that the leak volume corresponds to 0.16 litres of air per hour for a freeze dryer of 10 m^3 with a lyophilisation cycle time from the end of primary drying to stoppering of the vials of 25 hours, and the estimated CFU level can be calculated by:

$$\text{Contamination rate in freeze dryer} = \frac{\text{Leak in litres of air per hour x External air quality x Time}}{\text{Volume of freeze dryer}}$$

$$\text{CFU/m}^3\text{ in freeze dryer} = \frac{0.16\,\frac{\text{l}}{\text{hour}} \text{ x } 0.3 \text{ CFU/litre}^3 \text{ x } 25 \text{ hours}}{10\ \text{m}^3}$$

$$= 0.12\ \text{CFU/m}^3$$

$$0.5\ \mu\text{m particles/m}^3\text{ in freeze dryer} = \frac{0.16\,\frac{\text{l}}{\text{hour}} \text{ x } 220.2 \text{ particles/litre}^3 \text{ x } 25 \text{ hours}}{10\ \text{m}^3}$$

$$= 88\ 0.5\ \mu\text{m particles/m}^3$$

$$0.5\ \mu\text{m particles/m}^3\text{ in freeze dryer} = \frac{0.16\,\frac{\text{l}}{\text{hour}} \text{ x } 5.150 \text{ particles/litre}^3 \text{ x } 25 \text{ hours}}{10\ \text{m}^3}$$

$$= 2\ 5\ \mu\text{m particles/m}^3$$

Therefore, it can be shown that for a freeze dryer with an internal volume of 10 m^3 with a lyophilisation cycle time from the end of primary drying to stoppering of the vials of 25 hours, then a leak rate of 0.02 mbar L/s will provide Grade A air (0.12 CFU/m^3) during the lyophilisation cycle. For the same leak rate and cycle time, a freeze dryer of 1 m^3 would result in the Grade A air quality limit being exceeded (1.2 CFU/m^3) and the standard 0.02 mbar L/s leak rate limit would not be appropriate[2]. It can also be seen that for this analysis, assuming an external CFU contamination rate of 300 CFU/m^3 that the Grade A air quality specification would fail on CFU before the particulate levels were exceeded.

Visualisation of estimated maximum leak rate by cycle time and freeze dryer volume

Following the above mathematical analysis, a contour plot was developed to visualise the maximum leak rate that would ensure Grade A air conditions are met based on freeze dryer cycle time and volume (**Figure 4**). What this demonstrates, is that the maximum leak rate limit to maintain Grade A air conditions varies with the size of the freeze dryer and cycle time, and a single value of leak rate is not appropriate.

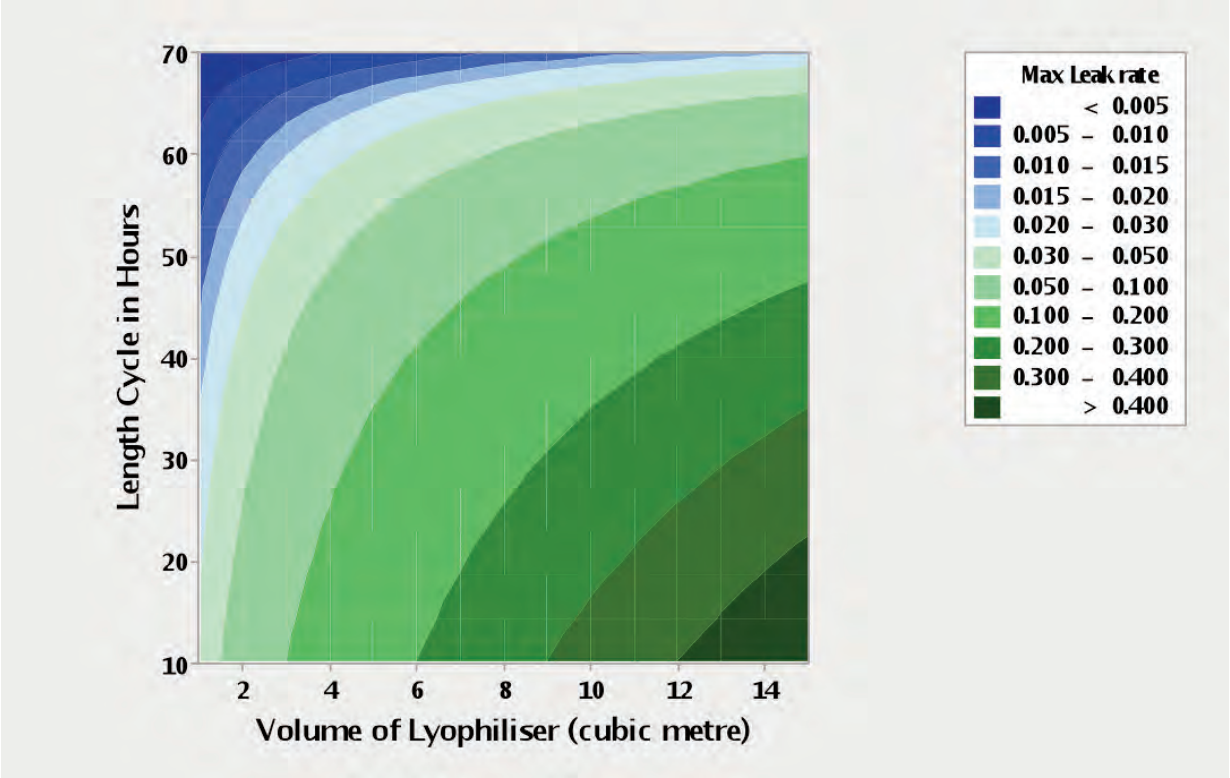

Figure 4. Visualisation of maximum leak rate in mbar L/s to ensure Grade A air quality is maintained by freeze dryer cycle and volume

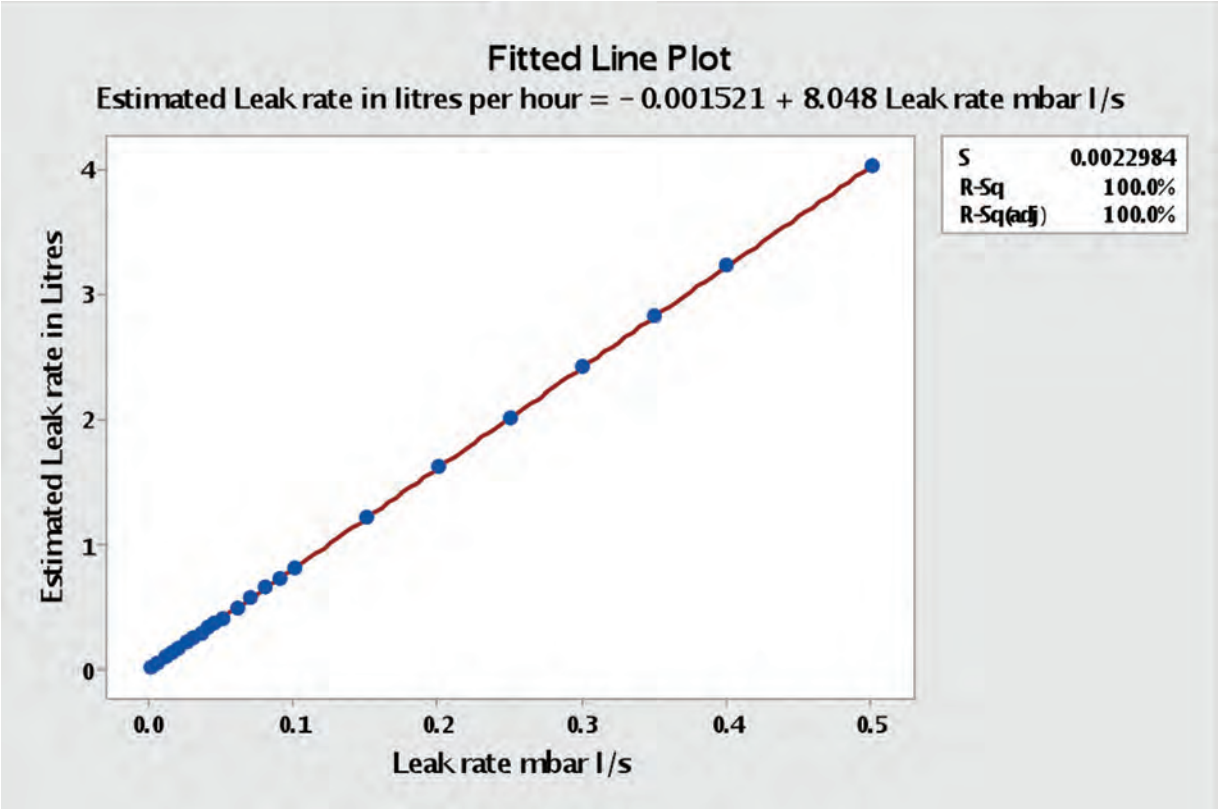

Figure 5. Estimated leak rate in litres per hour.

Estimation of leak in litres air per hour from leak rate in mbar L/s

The estimated leak rate in litres per hour is shown in **Figure 5**. To aid the user in calculating a leak rate in litres of air per hour from mbar L/s, the following formula may be used as an approximation.

Estimate leak rate in litres per hour = –0.001521 + 8.048 Leak rate mbar L/s

Therefore, a 0.02 mbar L/s leak rate can be estimated as giving a leak of 0.16 litres per hour and using the standard 300 CFU/m^3 external contamination rate this would give a contamination rate of 0.05 CFU/hour, which would allow a cycle time of 20 hours of lyophilisation in a 1 m^3 freeze dryer before Grade A air conditions were exceeded.

Conclusions and recommendations

This paper supports the approach undertaken by Hardwick *et al*.[3] with a simplified methodology for defining a scientific rationale for the establishment of acceptance criteria for leak rates in pharmaceutical freeze dryers based on the lyophilisation cycle and freeze dryer volume.

Calculations have been made to matrix the combination of cycle time and freeze dryer volume, using the worst-case assumptions of mechanism of contamination ingress, external air bioburden of 300 CFU/m^3 and NVP contamination. This allows the definition of an established leak rate limit for the freeze dryer/cycle combination to be able to maintain Grade A microbial standards for the length of its lyophilisation cycle/secondary drying. Even with all the worst-case assumptions, it can be shown that leak rates that are 10 to 20 times higher than the frequently quoted acceptable leak rate of 2 x 10^{-2} mbar L/s specification would maintain Grade A air conditions inside commercial scale freeze dryers.

It is recommended that this methodology is used as a justification for the setting of leak rate limits as defined in *Leak Testing of Freeze-Dryers*[2], for development of new cycles or purchase of new freeze dryers, to ensure an adequate leak rate pass limit is adequately assessed and defined. This becomes increasingly necessary as freeze dryer sizes increase beyond 15 m^3 because the ability to assess leak rates of 0.02 mbar L/s or less will be determined by the ability to measure very small pressure rises (~4 µbar over 1 hour) and will be limited by the process capability of the pressure sensors.

Furthermore, it is recommended that a freeze dryer process capability leak rate limit (for example 0.02 mbar L/s) is continued to be used following each sterilisation cycle to ensure that process capability of the freeze dryer is ensured, agreeing with the FDA guidance, "Should the leak rate exceed specified limits, determine the actual leak site for purposes of repair". However, as an aid to assess the potential microbial impact to any cycle that exceeds the post-test leak rate acceptance criteria, a methodology has been established to determine this risk.

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