CCIT Feasibility Study of COMPANY X Product Samples Using Rapid Non-Destructive Headspace Analysis

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Introduction

Non-destructive headspace inspection can be used in many ways to improve and control the quality of your production process. Different examples of applying non-destructive headspace analysis are given in the References at the end of this report. One of the important applications is the identification of vials that have lost container closure integrity during production or afterwards. In this report the headspace analysis of COMPANY X lyophilized product vials is described. The objective of this feasibility study was to determine which headspace analysis method is optimal for container closure integrity testing of the COMPANY X product.

Instrumentation and Method

Laser Absorption Spectroscopy: General Background Information

Laser absorption spectroscopy is an optical measurement method for rapid and non-invasive headspace gas analysis of sealed containers. The technique can measure a number of physical parameters within the headspace of a container, including gas concentrations and total headspace pressures.

The LIGHTHOUSE systems incorporate a high sensitivity detection method known as *frequency modulation spectroscopy* (FMS). A description of frequency modulation spectroscopy for laser-based headspace analysis is given below and schematically depicted in Figure 1.

Light from a near-infrared laser diode is tuned to match the internal absorption frequency of the target molecule (Figure 1, step I). The light is then passed through the headspace region of a container (Figure 1, step II), scanned in frequency and detected by a photodetector (Figure 1, step III). The amount of light absorbed is proportional to the target molecule concentration as can be seen in the graphical insert in Figure 1. Appendix A contains more details of the headspace oxygen, pressure, and moisture measurements.

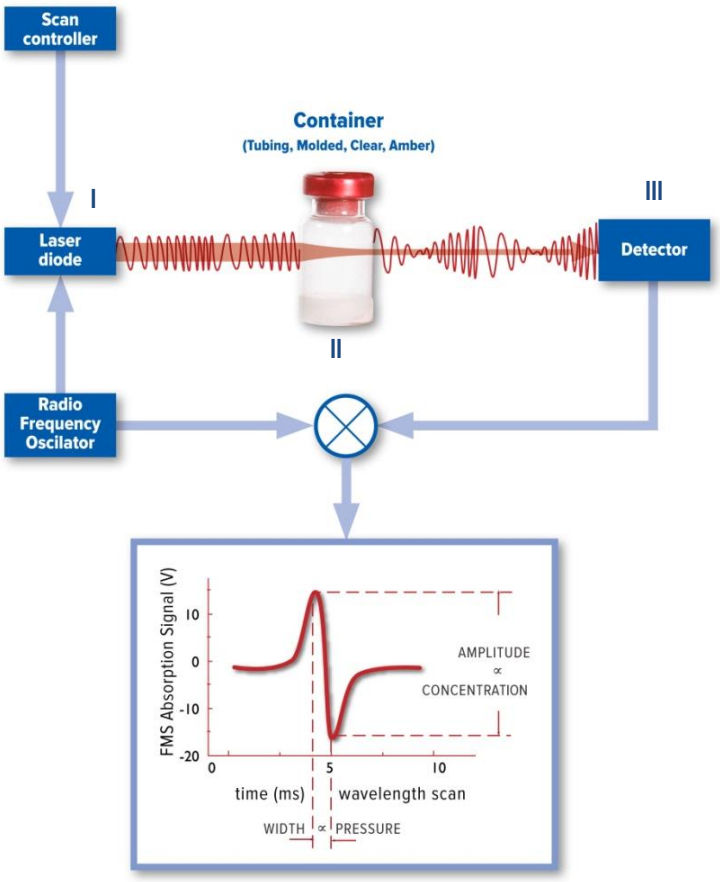

Figure 1: Schematic of frequency modulation spectroscopy for laser-based headspace analysis.

COMPANY X Sample Set

A total of twenty samples were provided by COMPANY X for headspace analysis. Sample set A consisted of ten 20R vials containing lyophilized product. Sample set B consisted of ten 50R vials containing lyophilized product.

Measurement Protocol

The objective of this feasibility study was to determine which headspace inspection method is optimal for container closure integrity testing of the COMPANY X product. In general, two headspace measurements can be used to determine if a vial has been leaking:

1. **Measurement of headspace pressure:** A vial that has leaked will be identified by the measurement of elevated headspace pressure (loss of vacuum).
2. **Measurement of headspace oxygen:** A vial that has leaked in an air environment will be identified by the measurement of oxygen that has leaked into the vial. This measured oxygen concentration can also be used to calculate the increase in headspace pressure as a result of the ingress of air into the vial.

Headspace Pressure measurements

Headspace pressure measurements were performed using a LIGHTHOUSE FMS-1400 Headspace Pressure/Moisture Analyzer (SN 275). Calibration was performed using certified NIST-traceable pressure standards manufactured by LIGHTHOUSE. Prior to sample analysis, a set of pressure standards was measured to verify performance of the analyzer for each vial size. After the analyzer performance was verified, each product sample was measured for headspace pressure.

Headspace Oxygen measurements

Headspace oxygen measurements were performed using a LIGHTHOUSE FMS-760 Headspace Oxygen Analyzer (SN 272). Calibration was performed using certified NIST-traceable oxygen standards manufactured by LIGHTHOUSE. Prior to sample analysis, a set of oxygen standards was measured to verify performance of the analyzer for each vial size. After the analyzer performance was verified, each product sample was measured for headspace oxygen.

Container closure integrity testing evaluation

After the initial headspace pressure and oxygen in all the samples were measured, one 20R sample was selected for a container closure integrity testing experiment. The conditions of a leak were simulated by incrementally injecting the sample with 0.2ml of air until the vial headspace reached atmospheric pressure. The headspace pressure and oxygen were measured five times after each injection to demonstrate the ability of the two measurement methods to detect a leaking sample.

Results

The headspace conditions of the samples as received from COMPANY X were first determined by performing headspace pressure and oxygen analysis. The following sections describe the results of these analyses. Appendix A lists all individual measurements and Appendix B gives information about the method (specificity, linearity, range, etc.).

Headspace pressure measurements

Prior to analysis of the headspace pressure levels in the product samples, a set of known pressure standards was measured to determine the performance of the headspace pressure analyzer for each vial size. Standards with known pressure levels were each measured five consecutive times. The mean measured headspace pressure and standard deviation of the standards are listed in Tables 1 and 2.

Table 1: Measurements of 20ml clear tubing glass vial pressure standards (N=5)

	Headspace pressure	
Label	Mean (mbar)	St. Dev. (mbar)
2.5 mbar standard	5.4	0.06
60.9 mbar standard	54.6	0.06
120.8 mbar standard	117.8	0.17
254.2 mbar standard	250.7	0.16
507.7 mbar standard	516.2	0.33
653.9 mbar standard	666.2	0.53
801.0 mbar standard	809.3	0.18
935.7 mbar standard	940.7	0.41

Table 2: Measurements of 50ml clear tubing glass vial pressure standards (N=5)

	Headspace pressure	
Label	Mean (mbar)	St. Dev. (mbar)
2.8 mbar standard	4.2	0.15
60.1 mbar standard	52.5	0.04
120.4 mbar standard	110.2	0.16
254.1 mbar standard	247.8	0.30
506.6 mbar standard	512.6	0.38
654.1 mbar standard	664.2	0.30
800.0 mbar standard	811.6	0.31
931.9 mbar standard	940.7	0.42

After measurement of the standards, the headspace pressure in each product sample was measured once. The measured headspace pressure levels are plotted in Figure 5.

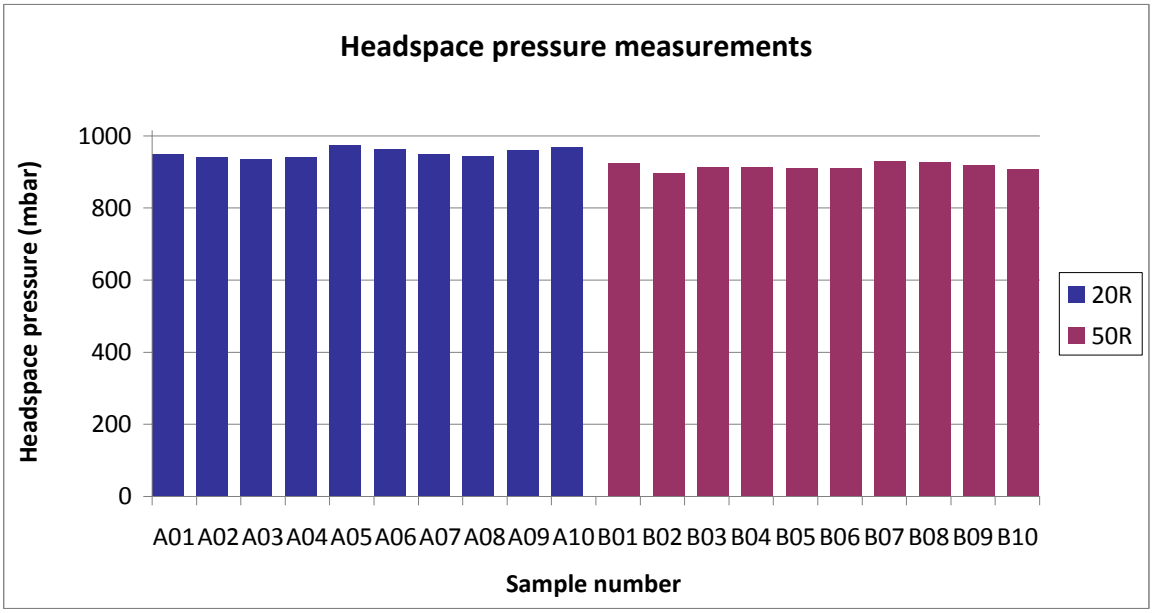

Figure 5: Measured headspace pressure of 20R and 50R vials containing lyophilized product

Headspace oxygen measurements

Prior to headspace oxygen analysis of the product samples, a set of known oxygen standards was measured to determine the performance of the headspace oxygen analyzer for each vial size. Standards with known oxygen concentrations were each measured five consecutive times. The mean measured headspace oxygen and standard deviation of the standards are listed in Tables 3 and 4.

Table 3: Measurements of 20ml clear tubing vial oxygen standards (N=5)

	Headspace oxygen	
Label	Mean (% atm)	St. Dev. (% atm)
20.0% standard	20.0	0.03
8.0% standard	8.0	0.09
4.0% standard	3.9	0.02
2.0% standard	2.0	0.02
1.0% standard	1.0	0.05
0.0% standard	0.0	0.04

Table 4: Measurements of 50ml clear tubing vial oxygen standards (N=5)

	Headspace oxygen	
Label	Mean (% atm)	St. Dev. (% atm)
20.0% standard	20.0	0.02
8.0% standard	8.1	0.08
4.0% standard	4.1	0.02
2.0% standard	2.0	0.04
1.0% standard	1.0	0.03
0.0% standard	0.0	0.03

After measurement of the standards, the headspace oxygen in each product sample was measured once. The measured headspace oxygen concentrations are plotted in Figure 6.

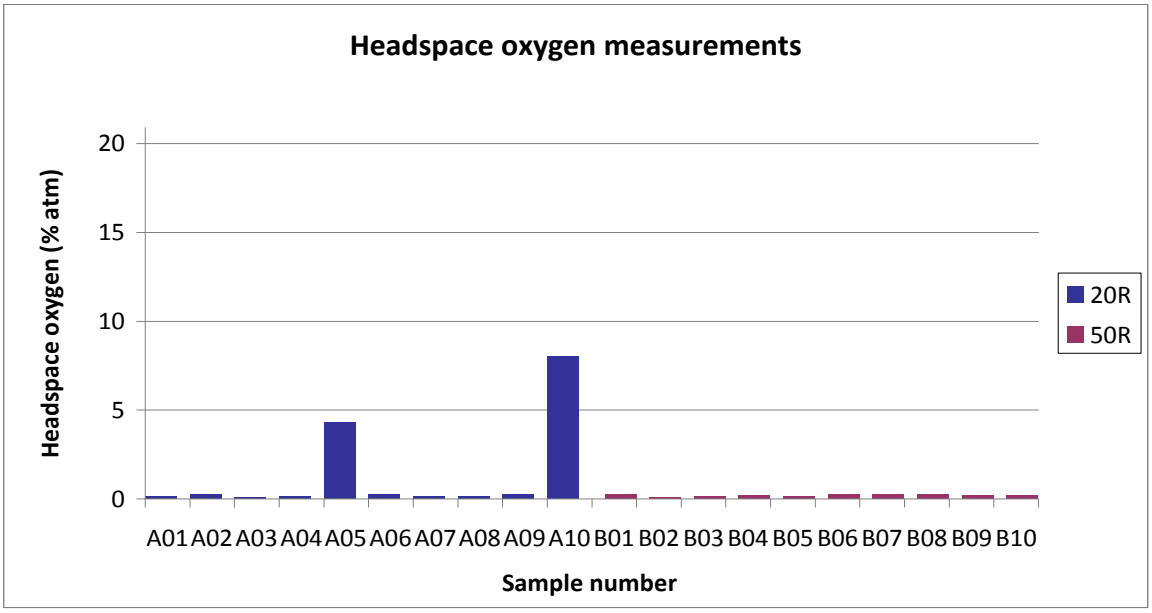

Figure 6: Measured headspace oxygen of Sample Set A (20R vials) and Sample Set B (50R vials).

Container closure integrity testing evaluation

One 20R vial sample (number A01) was selected for a container closure integrity testing experiment. The headspace pressure and oxygen were measured five consecutive times after each 0.2ml injection of air to demonstrate the ability of the systems to detect a sample which is experiencing a leak. The individual measurements are shown in Figures 7 and 8 below.

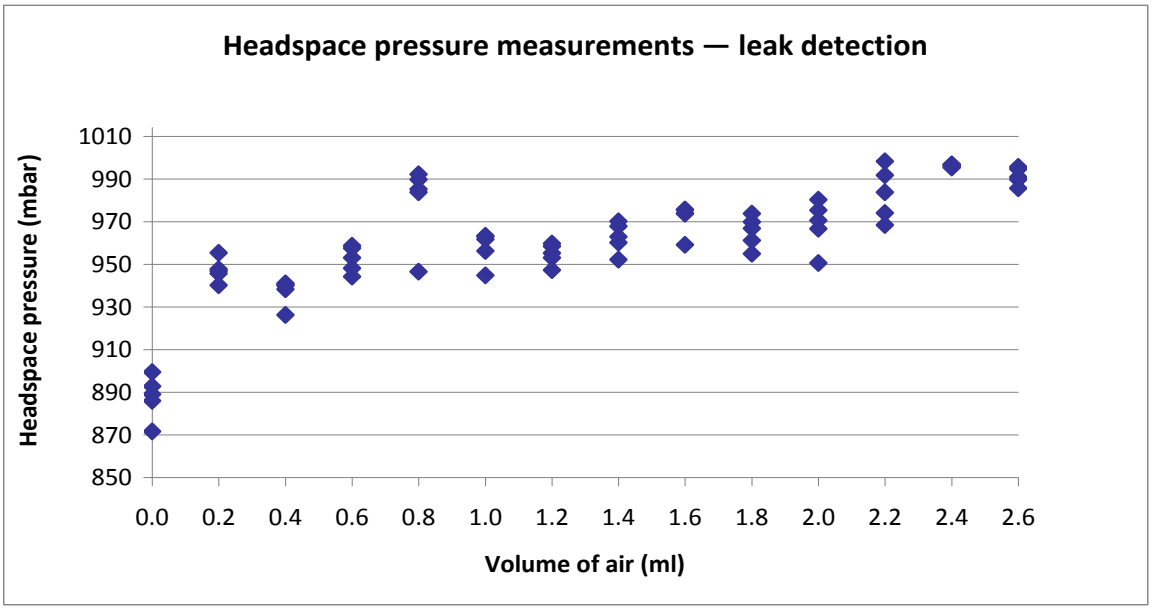

Figure 7: Individual headspace pressure measurements of a simulated leak in a 20R sample.

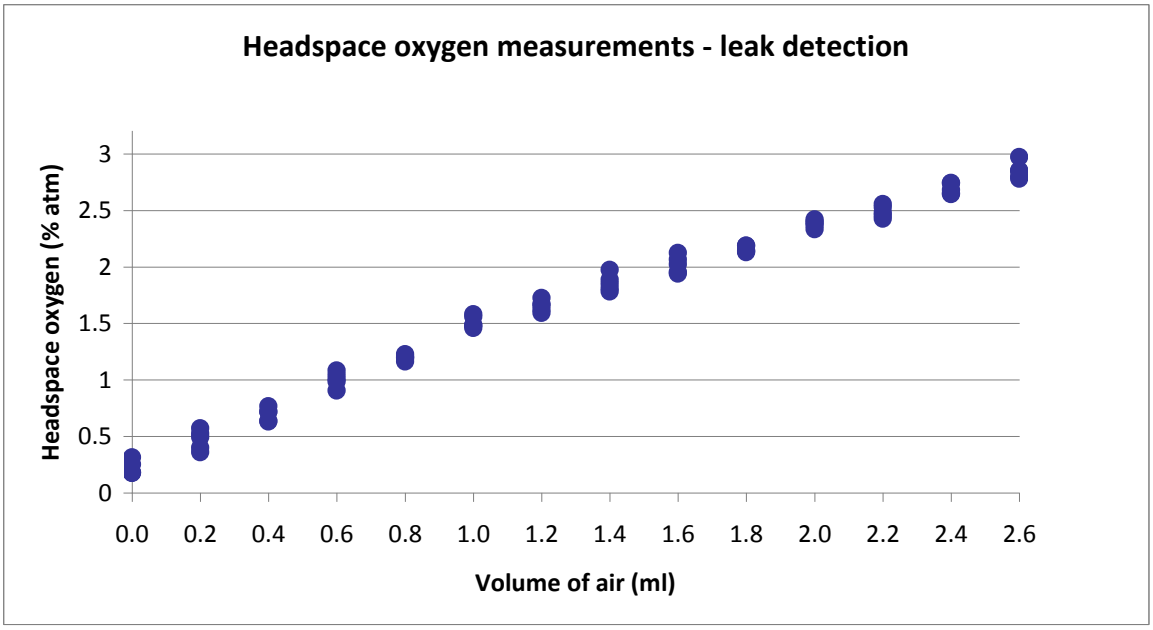

Figure 8: Individual headspace oxygen measurements of a simulated leak in a 20R sample.

In a real-world scenario for a product with these initial conditions, a leak would result in a rapid increase in the headspace oxygen concentration to about 2.6-2.8% as air is forced into the vial to fill the remaining vacuum. If that leak remains open for an extended period of time (for example, a crack in the glass) or if the leak is large enough before it is sealed (for example, a raised stopper) then the headspace oxygen concentration will have an opportunity to increase towards atmospheric levels (approximately 20.9%) due to diffusion of gas through the leak. This explains the elevated oxygen concentrations in samples A05 and A10 (identified as real leakers in this feasibility study), which are higher than the values reached in this leak simulation.

Discussion

The initial measurements characterize the headspace conditions to be near one atmosphere (approximately 850-900 mbar) with little or no oxygen in the headspace. Two samples (A05 and A10) were found to have elevated oxygen concentrations and slightly elevated pressures, indicating that these two samples had temporarily leaked after leaving the lyophilization chamber before they were capped. The capping and crimping then stopped the leaks.

The container closure integrity testing evaluation demonstrates how effective the two headspace measurement methods are for detecting a leak in this product. Due to the high initial headspace pressure — which results in a greater uncertainty in the pressure measurement — the pressure measurement is not as an effective method as the oxygen measurement for detecting leaks in this product as small changes in the headspace pressure are difficult to measure. However, the oxygen measurement is capable of detecting the sizeable increase in headspace oxygen when air leaks into the vial, providing an effective method for container closure integrity testing. The effectiveness of the oxygen measurement is confirmed by the measurements on samples A05 and A10, two real-world leaks that are difficult to identify based only on the pressure measurement, but are easily identifiable by the elevated headspace oxygen concentration.

Conclusions

For lyophilized product packaged at higher pressures (600 mbar and greater), the preferred measurement method for headspace container closure integrity testing is the oxygen measurement rather than the pressure measurement. The pressure measurement is better suited to inspecting product with lower headspace pressures as the vacuum loss due to a leak would be greater. The results of this study demonstrate that container closure integrity testing of the COMPANY X product can be performed most effectively using the headspace oxygen measurement. The headspace oxygen measurement clearly identified two real-world leaks in the sample set. The headspace oxygen measurement also clearly tracked the rise of oxygen levels in the simulated leak study performed as part of the feasibility study.

For these reasons, the recommended method for container closure integrity testing of the COMPANY X product is the headspace oxygen measurement.

References

For Further Reading

[1] LIGHTHOUSE White Paper: "Introduction to Laser-Based Headspace Inspection and the Application to 100% Container Closure Inspection of Sterile Pharmaceutical Containers"
Download at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Brochure_Whitepaper_CCI_2013_WEB.pdf

[2] LIGHTHOUSE Application Note 103: "Detecting Raised Stoppers in Sterile Freeze Dried Vials"
Download at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Raised_Stopper_Appnote_103_2013_WEB.pdf

[3] LIGHTHOUSE White Paper: "Using Laser-Based Headspace Moisture Analysis for Rapid Nondestructive Moisture Determination of Sterile Freeze-Dried Placebo"
Download at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Brochure_Whitepaper_Moisture_2009_2013_WEB.pdf

[4] Veale, J. "New Inspection Techniques for Aseptic Processing" Chapter 11 of Practical Aseptic Processing, Fill and Finish, Vol. 1, edited by Jack Lysfjord
Can be ordered at:
https://store.pda.org/

Appendix A

This Appendix gives more detail about the laser-based headspace measurement.

Headspace Oxygen Measurement Principle

The LIGHTHOUSE FMS-760 Headspace Oxygen Analyzer operates on the principles of frequency modulation spectroscopy (FMS) as described earlier. Light from a near infrared diode laser is directed through the headspace region of a sealed (parenteral) container. Since oxygen absorbs near infrared light in a band of transitions centered at 762 nm, the LIGHTHOUSE FMS-760 diode laser operates at this wavelength.

The amount of laser light absorbed by an individual transition in the oxygen A-band is proportional to the oxygen concentration in the headspace of a container. During a measurement, the laser frequency is repeatedly scanned over the absorption feature and successive scans are averaged to improve the signal to noise ratio. As can be seen from the graph depicted in Figure 2, the averaged light absorption signal is proportional to the headspace oxygen concentration.

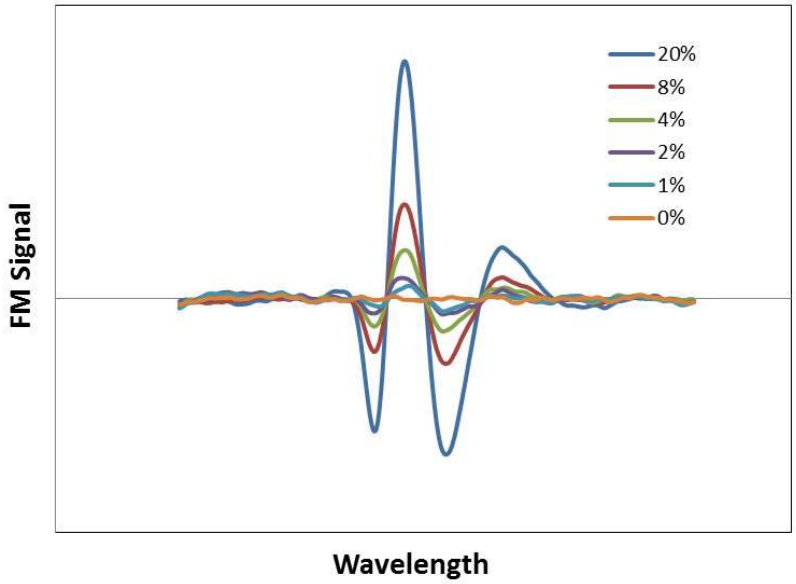

Figure 2: Frequency modulation signals from oxygen absorption in 10mL ampoules filled with certified gas mixtures of oxygen in nitrogen. The peak-to-peak amplitude of each spectrum is proportional to the oxygen concentration.

Headspace Pressure and Moisture Measurement Principle

The LIGHTHOUSE FMS-1400 Headspace Pressure/Moisture Analyzer also operates on the previously described frequency modulation spectroscopy principles. The laser diode of an FMS-1400 is tuned to match the frequency of moisture molecules at 1400 nm. The FMS-1400 returns a value for headspace pressure *and* moisture from a single measurement. This is possible since the width of the absorption signal is proportional to the headspace pressure, whilst the area is proportional to the headspace water vapor levels.

In Figure 3 a set of spectra taken at different headspace pressures is shown. It can be seen from these spectra that the signal is wider at atmospheric pressure (top line) than at 0.04 atm (bottom line). Measurement of this pressure broadening of the water absorption spectra enables the determination of the total pressure inside a sealed container. The spectra depicted in Figure 4 were taken from samples having different headspace moisture levels while keeping the headspace pressure level constant. It can be seen that a decrease in water vapor pressure in the headspace results in a decrease in area of the signal.

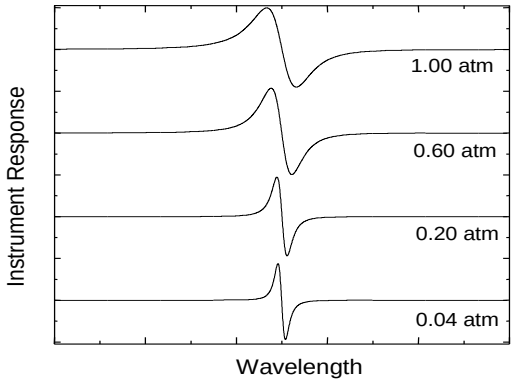

Figure 3: Absorption spectra for several known headspace pressures. Note that the signal width varies as a function of pressure.

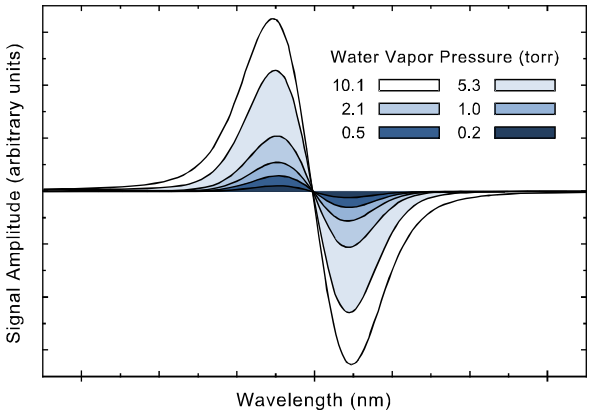

Figure 4: Absorption spectra for several known headspace moistures levels at constant background pressure.

Appendix B

This Appendix contains information about the analytical measurement method. The key to validating and challenging the performance of the headspace method is the availability of known traceable headspace standards. LIGHTHOUSE has in-house capabilities for manufacturing NIST traceable headspace standards. The standards are delivered with a certificate and are manufactured from empty vials supplied by the customer (see Figure 9) – therefore, from a glass configuration point-of-view the standards are identical to the product vials that will be measured by the headspace sensor. These NIST traceable standards are used to calibrate the headspace measurement system and are also used to challenge the performance of the headspace measurement system.

Figure 9: A NIST-traceable certified headspace standard manufactured by LIGHTHOUSE.

Performance data of LIGHTHOUSE headspace sensors has been collected by measuring thousands of NIST traceable standards made from all different types of pharmaceutical vials, ampoules, and syringes. LIGHTHOUSE always produces specific performance data for a customer using the customer containers to make NIST traceable standards. Those specific standards are then used to test and challenge the performance of the headspace system to be delivered. Performance data is generated during the configuration of a headspace system to ensure that the system meets the customer measurement requirements.

For this Feasibility Report, information was requested about the following performance parameters:

- **Specificity**

"The ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc."

The LIGHTHOUSE method has very high specificity because it is based on laser spectroscopy. The output of the laser is tuned to a single absorption line of the target molecule – only the target molecule will absorb light at the laser wavelength and produce a signal.

- **Linearity**

"The ability of an analytical procedure to obtain test results that are directly proportional to the concentration of analyte in the sample. "

All LIGHTOUSE systems are optimized for linear response. Below is an example showing linearity of the pressure measurement from 0 to approximately 1 atmosphere of pressure (760 torr):

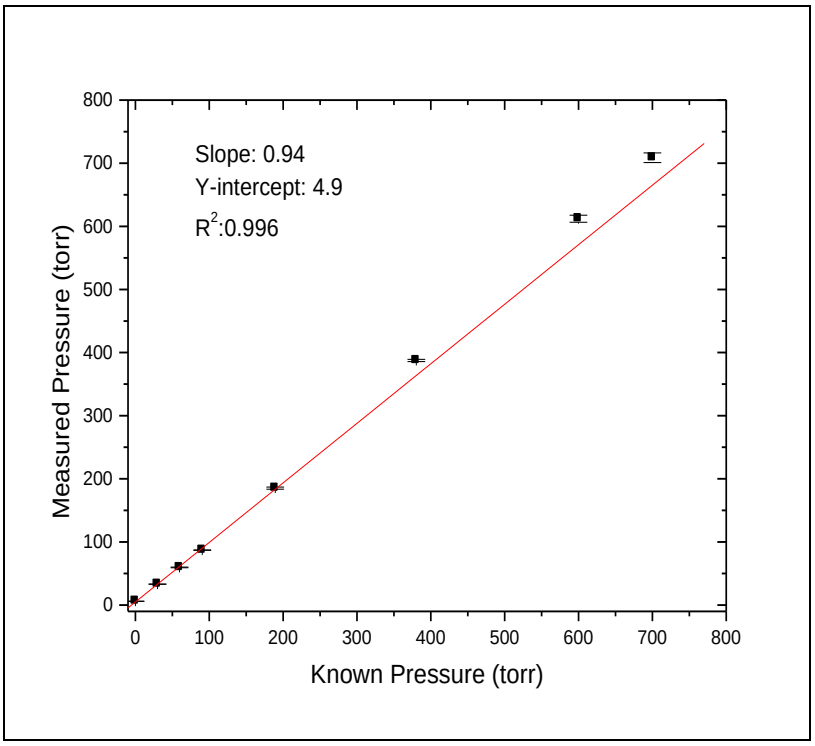

Figure 8 Linearity of pressure measurements on a 10cc vial.

- **Range**

"The interval from the upper to the lower concentration of analyte in the sample for which it has been demonstrated that the analytical method has suitable level of precision, accuracy & linearity."

As explained in this report, the headspace pressure or oxygen measurement can be used to detect a leaking lyophilized vial. Feasibility studies will determine which method has suitable precision, accuracy, and linearity over the full headspace parameter range. In this specific case for COMPANY X product, the headspace oxygen measurement has suitable precision, accuracy, and linearity over a headspace oxygen range that easily and clearly identifies a leaking vial.

- **Intermediary precision (ICH = ruggedness, USP = reproducibility)**

ICH lays down that Intermediate Precision is to cover the various influences within a laboratory, i.e. conducting analyses on two different (or several) days by different laboratory staff members, with different equipment (if available), etc. This is to examine in accordance with ICH Guideline Q2A the

effects of random events on the precision of an analytical method. Intermediate Precision therefore gives a first indication already of the future transferability of an analytical method.

LIGHTHOUSE has headspace laboratories in Virginia, USA and in Amsterdam, The Netherlands. Both laboratory locations have implemented and validated headspace systems to deliver Standards Re-certification Services. The performance data generated at both locations for these services is identical indicating a high level of intermediary precision (ruggedness, reproducibility).

- **Repeatability**

Repeatability: Within the same lab within a short time period, same analyst and same equipment

LIGHTHOUSE has run repeatability experiments at each of the LIGHTHOUSE headspace labs. The repeatability is high (within the standard deviation of the measurement – see example Performance Tables in this report).

- **Limit of quantitation**

"The lowest amount of analyte in a sample which can be quantitatively be determined with suitable precision and accuracy."

Typically the limit of quantitation is defined to be some multiple (definitions can vary) of the measurement standard deviation of a blank (zero standard). The performance tables can be used to determine a limit of quantitation:

- **Accuracy**

"The error of the measurement (mean minus actual)"

See above example Performance Table.

- **Robustness**

"A measure of the capacity of the procedure to remain unaffected by small, but deliberate variations in method parameters."

Two method parameters that can affect the measurement performance are measurement time and purge rate (LIGHTHOUSE headspace systems have a small purge of dry nitrogen/compressed air in the measurement region to get rid of atmospheric influences on the measurement). A purge rate is specified for the measurement system of 3 SLPM (standards liters per minute) of dry nitrogen/compressed air. Measurement performance will remain constant and robust for purge rates between 2 and 4 SLPM. Feasibility studies will determine a minimum measurement time that meets the robustness requirement.