# Non-Destructive Headspace Oxygen Analysis of Lyo Product Vials For Container Closure Integrity Determination

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**PREPARED FOR:**
Company X

LIGHTHOUSE
The Science of Pharmaceutical Manufacturing

## INTRODUCTION

Laser-based headspace analysis from LIGHTHOUSE allows for the rapid non-destructive analysis of headspace oxygen levels in parenteral containers (vials, ampoules, syringes, bottles, pouches). Headspace analysis can be used to assess if the container closure integrity of freeze-dried vials has been compromised, which would result in an increase in oxygen levels.

## 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.

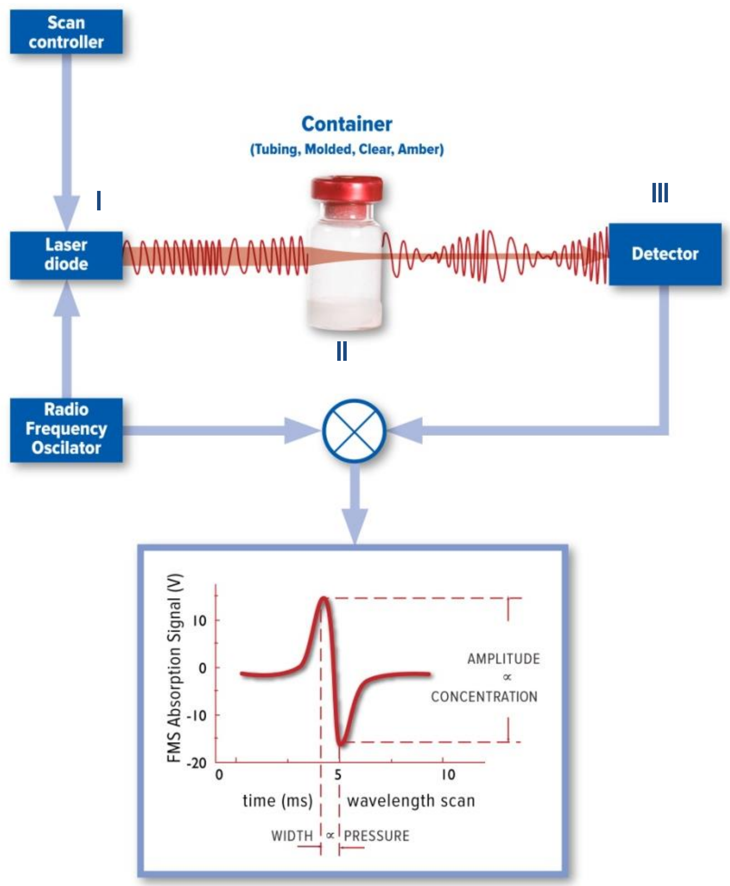


**Figure 1.** Schematic of frequency modulation spectroscopy for laser-based headspace analysis.

## 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.

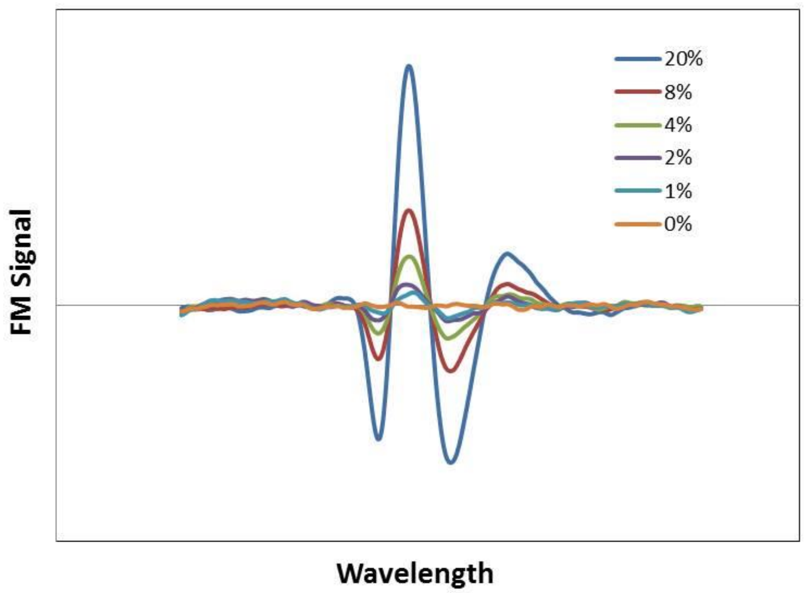


**Figure 2.** Frequency modulation signals from oxygen absorption in 10mL ampoules filled with certified gas mixtures of oxygen in nitrogen. The peak-to-peak amplitude of each spectrum is proportional to the oxygen concentration.

## INSTRUMENT PERFORMANCE AND METHOD VALIDATION

A set of flame-sealed oxygen standards manufactured by LIGHTHOUSE and traceable to the National Institute of Standards and Technology (NIST) was measured to determine the instrument performance for the two container sizes used in this study. Headspace oxygen measurements were performed using a Lighthouse Instruments FMS-760 Headspace Oxygen Analyzer.

Calibration was performed using NIST-traceable standards at 0% and 20% oxygen concentration. The headspace oxygen in each standard of both the 30ml (250mg) and 50ml (750mg) vial sizes was measured twenty times. The summary of results is presented in Tables 1 and 2 below.

**Table 1**. Performance data for 30ml clear tubing vials using standard set A001-30A (N=20).

| Standard Label | Actual (% $O_2$) | Mean (% $O_2$) | Error (% $O_2$) | St Dev (% $O_2$) | Min (% $O_2$) | Max (% $O_2$) |
|---|---|---|---|---|---|---|
| A001-30A-20 | 20.10 | 20.05 | -0.05 | 0.04 | 19.98 | 20.12 |
| A001-30A-8 | 8.01 | 7.92 | -0.09 | 0.04 | 7.83 | 8.01 |
| A001-30A-4 | 4.04 | 4.03 | -0.01 | 0.05 | 3.96 | 4.12 |
| A001-30A-2 | 2.00 | 1.95 | -0.05 | 0.04 | 1.87 | 2.03 |
| A001-30A-1 | 1.01 | 1.02 | 0.01 | 0.04 | 0.95 | 1.09 |
| A001-30A-0 | 0.00 | 0.02 | 0.02 | 0.03 | 0.00 | 0.08 |

**Table 2.** Performance data for 50ml clear tubing vials using standard set A001-50A (N=20).

| Standard Label | Actual (% $O_2$) | Mean (% $O_2$) | Error (% $O_2$) | St Dev (% $O_2$) | Min (% $O_2$) | Max (% $O_2$) |
|---|---|---|---|---|---|---|
| A001-50A-20 | 20.00 | 20.02 | 0.02 | 0.04 | 19.92 | 20.09 |
| A001-50A-8 | 8.01 | 7.97 | -0.04 | 0.03 | 7.92 | 8.05 |
| A001-50A-4 | 4.04 | 3.93 | -0.11 | 0.04 | 3.84 | 3.99 |
| A001-50A-2 | 2.00 | 1.88 | -0.12 | 0.04 | 1.81 | 1.97 |
| A001-50A-1 | 1.01 | 0.91 | -0.10 | 0.04 | 0.82 | 1.00 |
| A001-50A-0 | 0.00 | 0.08 | 0.08 | 0.05 | 0.01 | 0.17 |

The performance data are used to determine the following performance parameters: accuracy, precision, measurement uncertainty, limit of detection, and linearity. The accuracy is defined as the maximum difference of the mean from the actual value for a single standard within a set (i.e. the maximum absolute error). The precision is defined as the maximum standard deviation of any one standard in a set. The measurement uncertainty represents the sum of the accuracy and precision. The limit of detection is defined as three times the mean measurement of the 0% standard. The linearity is defined by the linear regression fit applied to a plot of measured values versus actual values (see Figures 3 and 4). A summary of these performance parameters is outlined in Table 3.

**Table 3.** Assessment of FMS performance parameters in 30ml and 50ml oxygen standards.

| | | 30ml vial | 50ml vial |
|---|---|---|---|
| Accuracy | (% atm) | 0.09 | 0.12 |
| Precision | (% atm) | 0.05 | 0.05 |
| Uncertainty | (% atm) | 0.13 | 0.17 |
| Limit of Detection | (% atm) | 0.06 | 0.24 |
| Linearity | Slope | 0.997 | 1.003 |
| | R-squared | 1.0000 | 0.9999 |
| | y-intercept | 0.011 | 0.065 |

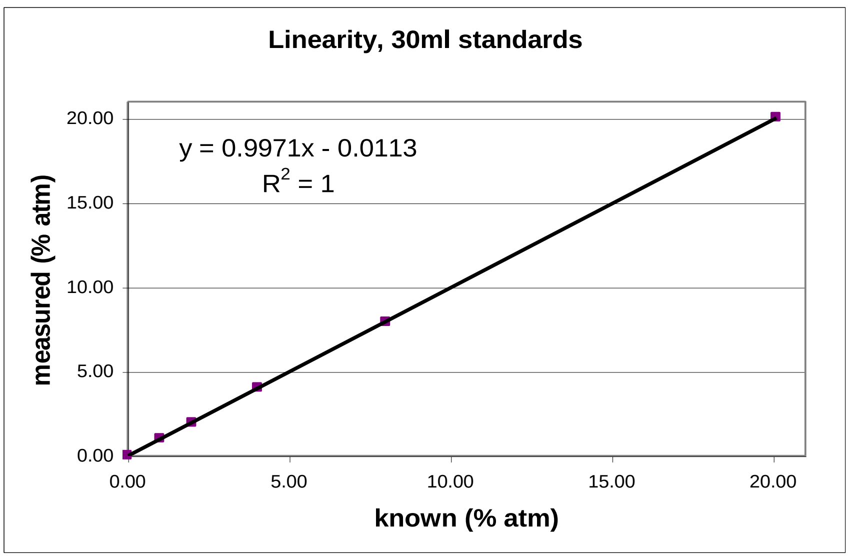


**Figure 3.** Linearity of measurements on 30ml oxygen standards.

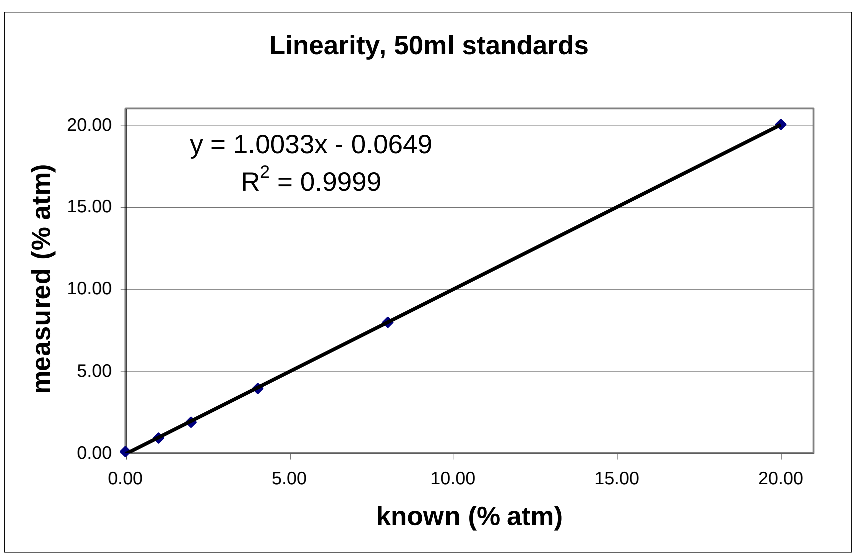


**Figure 4.** Linearity of measurements on 50ml oxygen standards.

## SAMPLE SET

One thousand five hundred (1500) vials containing lyophilized product were provided for analysis of headspace oxygen concentration. The samples consisted of several different product vial configurations. These are listed below:

**Sample Set 1**: Five hundred 30cc clear tubing vials containing placebo, packaged under 150 torr of nitrogen.

**Sample Set 2**: Five hundred 30cc clear tubing vials containing Product Y, packaged under 150 torr of nitrogen.

**Sample Set 3**: Five hundred 50cc clear tubing vials containing Product Z, packaged under 26 torr of nitrogen.

## MEASUREMENT PROTOCOL

### Oxygen Analysis

Headspace oxygen measurements were performed using a Lighthouse Instruments FMS-760 Headspace Oxygen Analyzer (sn A001). Calibration was performed using NIST-traceable standards sets A001-30A (30cc) and A001-50A (50cc) manufactured by Lighthouse Instruments. The headspace oxygen in each sample was measured one time. Samples that displayed elevated headspace oxygen concentration were subjected to repeated measurements. The results are presented in the following section.

### Calculating Air Ingress

Air ingress to the vials was calculated using the headspace oxygen measurement results. Headspace pressure was estimated using the theoretical leak curves, which correlate FMS-760 oxygen measurements to headspace pressures. These values are based on the assumption that all samples begin at their target fill pressure of nitrogen and leak only air.

## RESULTS

### Sample Set 1: Placebo

Results of the oxygen measurements on all samples are displayed graphically in Figure 5. Of the 500 samples measured, the majority (482) showed measured oxygen concentrations less than 3%. Measurement statistics and calculated headspace pressures of the samples are presented in Table 4. A total of 28 samples showed elevated headspace oxygen levels.

**Figure 5.** Headspace oxygen measurements for Sample Set 1.

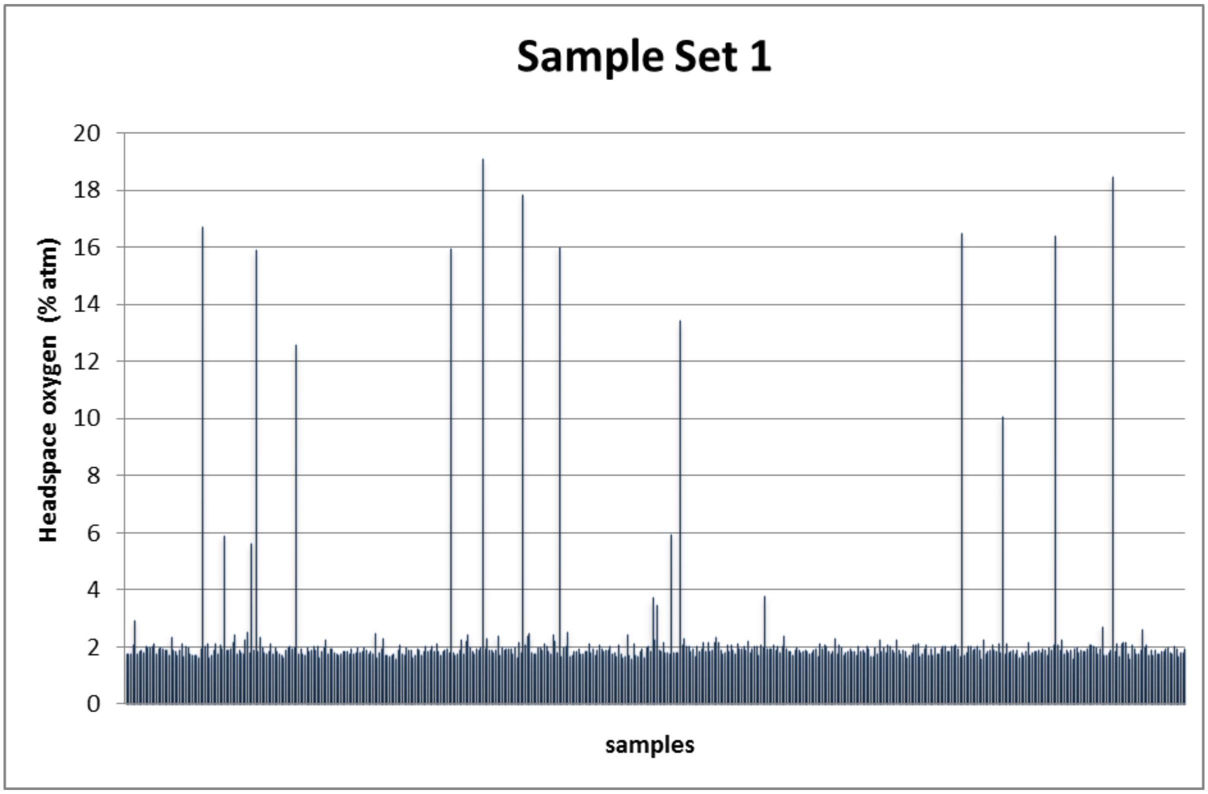

**Table 4.** Measurement statistics for Sample Set 1. The data under "Measurements < 3% oxygen" represent the mean, standard deviation, maximum value, and minimum value for all measurements on samples that measured less than 3% oxygen. The data under "Measurements > 3% oxygen" are the mean and standard deviation of 3 measurements on each sample with measured headspace oxygen concentration above 3%. The calculated pressures are based on the mean oxygen values.

Measurement < 3% oxygen
482 vials, 1 measurement each

| Mean (% atm) | Stdev (% atm) | Max (% atm) | Min (% atm) | Calculated Pressure (torr) |
|---|---|---|---|---|
| 1.93 | 0.18 | 2.94 | 1.59 | 164 |

Measurement > 3% oxygen
18 vials, 3 measurements each

| Sample | Mean (% atm) | Stdev (% atm) | Calculated Pressure (torr) |
|---|---|---|---|
| 37 | 16.63 | 0.07 | > 348 |
| 47 | 5.96 | 0.01 | 197 |
| 60 | 5.60 | 0.03 | 194 |
| 62 | 15.75 | 0.03 | > 327 |
| 81 | 12.45 | 0.05 | 270 |
| 154 | 15.80 | 0.02 | > 328 |
| 169 | 18.89 | 0.03 | > 433 |
| 188 | 17.66 | 0.05 | > 378 |
| 205 | 15.88 | 0.04 | > 330 |
| 249 | 3.65 | 0.06 | 177 |
| 251 | 3.44 | 0.03 | 176 |
| 258 | 5.88 | 0.03 | 196 |
| 262 | 13.34 | 0.02 | 283 |
| 302 | 3.68 | 0.02 | 177 |
| 395 | 16.40 | 0.03 | > 342 |
| 414 | 9.95 | 0.04 | 238 |
| 439 | 16.33 | 0.01 | > 341 |
| 466 | 18.26 | 0.04 | > 401 |

**Sample Set 2: Product Y**

Results of the oxygen measurements on all samples are displayed graphically in Figure 6. Of the 500 samples measured, the majority (497) showed measured oxygen concentrations less than 1.5%. Measurement statistics and calculated headspace pressures of the samples are presented in Table 5. A total of three samples showed elevated headspace oxygen levels.

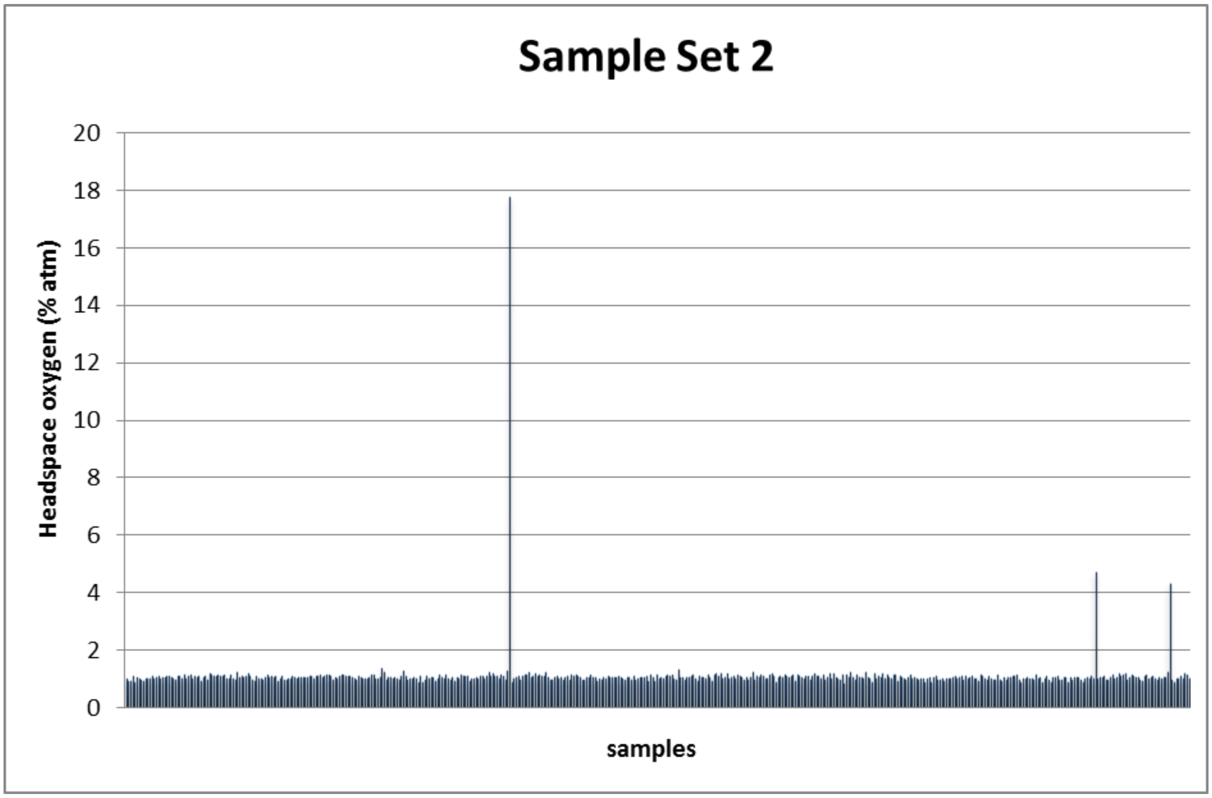


**Figure 6.** Headspae oxygen measurements for Sample Set 2.

**Table 5.** Measurement statistics for Sample Set 2. The data under "Measurements < 1.5% oxygen" represent the mean, standard deviation, maximum value, and minimum value for all measurements on samples that measured less than 1.5% oxygen. The data under "Measurements > 1.5% oxygen" are the mean and standard deviation of 3 measurements on each sample with measured headspace oxygen concentration above 1.5%. The calculated pressures are based on the mean oxygen values.

Measurement < 1.5% oxygen
497 vials, 1 measurement each

| Mean (% atm) | Stdev (% atm) | Max (% atm) | Min (% atm) | Calculated Pressure (torr) |
|---|---|---|---|---|
| 1.06 | 0.07 | 1.28 | 0.84 | 158 |

Measurement > 1.5% oxygen
3 vials, 3 measurements each

| Sample | Mean (% atm) | Stdev (% atm) | Calculated Pressure (torr) |
|---|---|---|---|
| 181.00 | 17.45 | 0.02 | > 371 |
| 456.00 | 4.63 | 0.03 | 185 |
| 491.00 | 4.21 | 0.04 | 182 |

**Sample Set 3: Product Z**

Results of the oxygen measurements on all samples are displayed graphically in Figure 7. Of the 500 samples measured, all showed measured oxygen concentrations less than 1.6%. Measurement statistics and calculated headspace pressures of the samples are presented in Table 6.

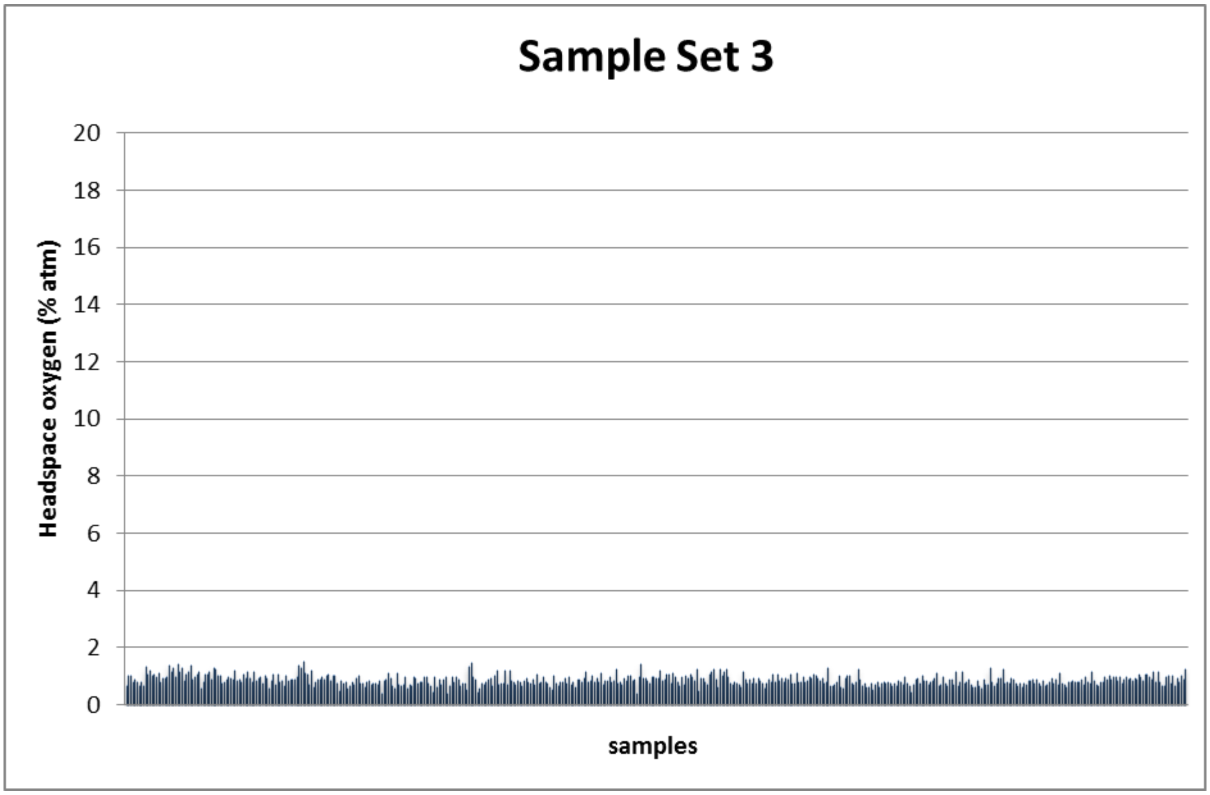


**Figure 7.** Headspace oxygen measurements for Sample Set 3.

**Table 6.** Measurement statistics for Sample Set 3. The calculated pressures is based on the mean oxygen value.

| Mean (% atm) | Stdev (% atm) | Max (% atm) | Min (% atm) | Calculated Pressure (torr) |
|---|---|---|---|---|
| 0.88 | 0.18 | 1.51 | 0.39 | 30 |

## Discussion

The results presented in this report show that measurement of headspace oxygen was possible with the lyo product vials provided by Company X. The measured headspace oxygen concentrations identify vials that have experienced a leak. If a lyo product vial sealed under a partial pressure of nitrogen experiences a leak, air will ingress into the container resulting in elevated levels of headspace oxygen. Leaking vials were identified in Sample Set 1 and Sample Set 2. Some leaking vials display near atmospheric headspace oxygen levels meaning that these vials have experienced a full exchange of gas between the initial headspace and the outside environment due to a permanent leak. Other leaking vials show partially elevated headspace oxygen levels meaning that these vials have suffered from a partial temporary leak. It is assumed that these partial leakers were leaking coming out of the lyo chamber and were then sealed shortly afterwards by the capping and crimping process after having partially leaked.

## Conclusions

The ability to rapidly and nondestructively measure headspace oxygen levels enabled insight into the container closure integrity of the samples.

The results presented here enable the following conclusions:

- There were leaking vials in Sample Set 1 and Sample Set 2.
- No leaking vials were found in Sample Set 3.
- Leaking vials having near atmospheric oxygen levels have fully leaked due to a permanent leak resulting in full gas exchange between the headspace and the outside environment.
- There are also leaking vials that partially leaked. These vials leaked between the lyo chamber and the capping and crimping machine. The capping and crimping process sealed these vials resulting in a leak that was partial and temporary.