# Rapid Non-Destructive Headspace Analysis of Lyo Product Vials

**PREPARED FOR:**

Company X

**PREPARED BY:**

Suzanne Kuiper, PhD, Application Scientist
email: skuiper@lighthouseinstruments.com

**REVIEWED BY:**

Derek Duncan, PhD, Product Line Manager
LIGHTHOUSE Instruments, LLC.
e-mail: dduncan@lighthouseinstruments.com

www.lighthouseinstruments.com

LIGHTHOUSE

# Introduction

Non-destructive headspace inspection can be used to quantitatively characterize headspace conditions. Headspace analysis can be used to assess if the container closure integrity of freeze-dried vials has been compromised, which would result in loss of vacuum or an increase in oxygen levels.

Elevated pressures can indicate a loss of vacuum through a leak in the container. In addition, this measurement also returns a value for the headspace moisture levels present in the vial. Headspace moisture values can, in many cases, be correlated to the moisture determination values obtained using Karl Fischer titration, or can even be correlated directly to the product stability.

# 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.

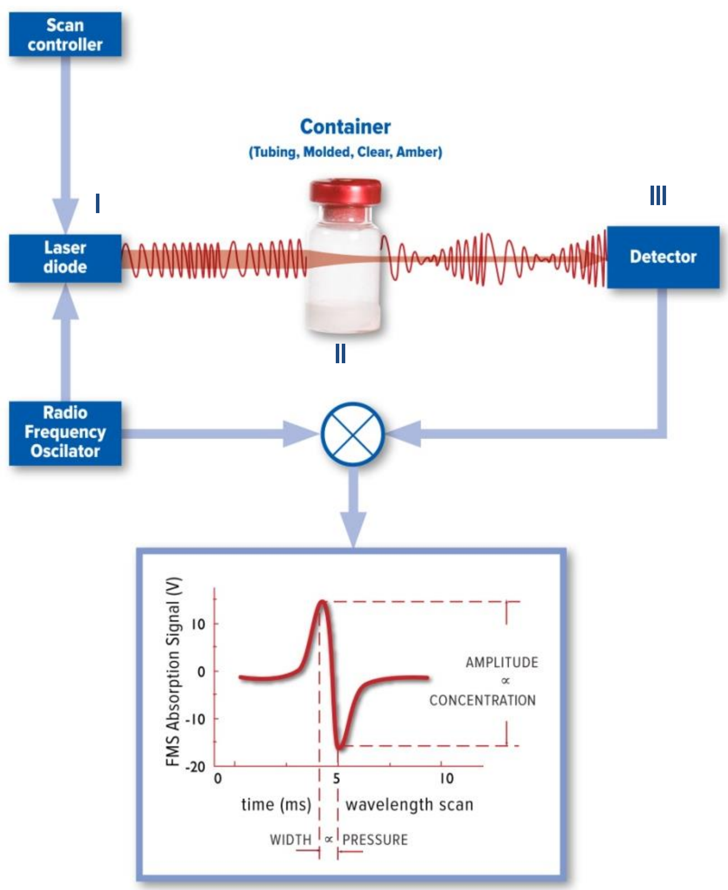


**Figure 1.** Schematic of frequency modulation spectroscopy for laser-based headspace analysis.

## Headspace Oxygen Measurements

### 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.

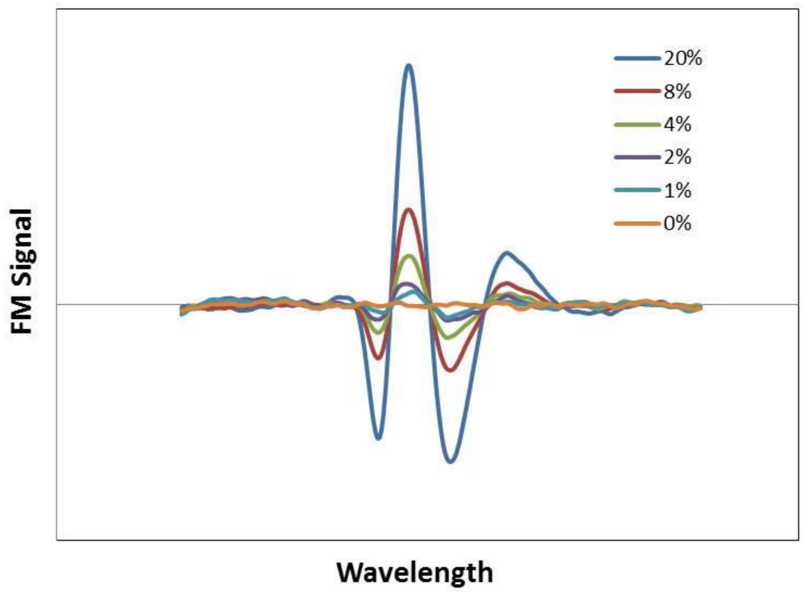


**Figure 2.** Frequency modulation signals from oxygen absorption in 10mL ampoules filled with certified gas mixtures of oxygen in nitrogen. The peak-to-peak amplitude of each spectrum is proportional to the oxygen concentration.

# Headspace Pressure and Moisture Measurements

### 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). 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.

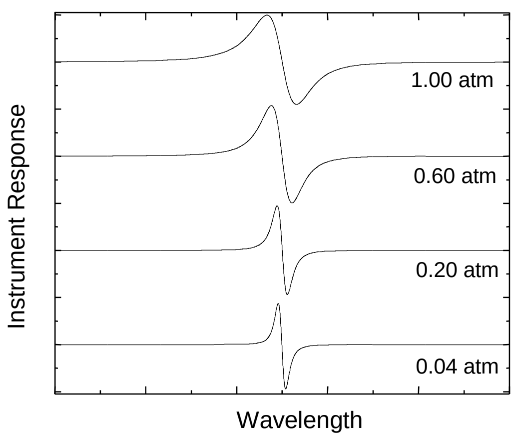


**Figure 3:** Absorption spectra for several known headspace pressures. Note that the signal width varies as a function of pressure.

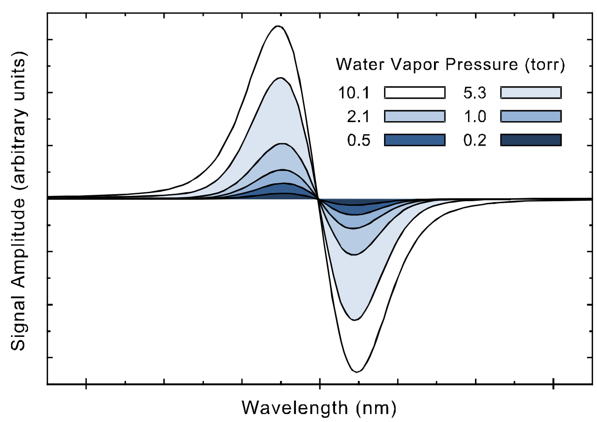


**Figure 4:** Absorption spectra for several known headspace moistures levels at constant background pressure.

## Sample Set

For this particular project 42 vials containing freeze dried product were provided for analysis of headspace oxygen, pressure, and moisture levels. The samples were divided over seven separate sets, each set containing six different vials.

The aim of this study was to investigate the feasibility of using headspace inspection to determine container closure integrity by distinguishing between varying levels of headspace pressure and oxygen. A secondary objective was to demonstrate the analysis of headspace moisture levels in the lyophilized product vials.

## Measurement Protocol

**Headspace Oxygen measurements**

Headspace oxygen measurements were performed using a validated LIGHTHOUSE FMS-760 Headspace Oxygen Analyzer. The instrument was turned on and allowed to warm up for thirty minutes. Calibration was performed using certified NIST-traceable oxygen standards manufactured by LIGHTHOUSE.

Prior to sample analysis, six oxygen standards (at 20, 8, 4, 2, 1, and 0% oxygen) were each measured three consecutive times to verify performance of the analyzer.

**Headspace Pressure/Moisture measurements**

Headspace pressure and moisture measurements were performed using a validated LIGHTHOUSE FMS-1400 Headspace Pressure/Moisture Analyzer. The instrument was turned on and allowed to warm up for thirty minutes. Calibration was performed with known pressure standards. Prior to sample analysis, five pressure standards (at 41, 81, 122, 253, and 506 mbar) were each measured three consecutive times to verify performance of the analyzer.

The results are presented and discussed in the following sections of this report.

***NOTE:*** The ability to make accurate headspace pressure measurements in the vials depends on the amount of headspace water vapor present. If there is enough water vapor to make a pressure measurement in a vial, the accuracy will be approximately ±20 mbar or ±15% of the absolute pressure value measured, whichever is greater.

# Results

### Headspace Pressure Results

Prior to analysis of the product vials, a set of known pressure standards was measured to determine the performance of the headspace analyzer. The standards of known pressure levels were each measured three consecutive times. The mean measured headspace levels and the corresponding standard deviations are listed in Table 1.

**Table 1:** Measurements of known pressure standards

| | Headspace pressure | |
|---|---|---|
| **Label** | **Mean (mbar)** | **St. Dev. (mbar)** |
| 41 mbar standard | 44 | 1.04 |
| 81 mbar standard | 82 | 1.07 |
| 122 mbar standard | 119 | 0.03 |
| 253 mbar standard | 270 | 2.41 |
| 506 mbar standard | 542 | 1.06 |

The measured headspace pressure levels in the lyo product vials are plotted in the Figure 5 for visual representation. The results are plotted per set of six samples. The individual measurement results can be found in the appendix of this report.

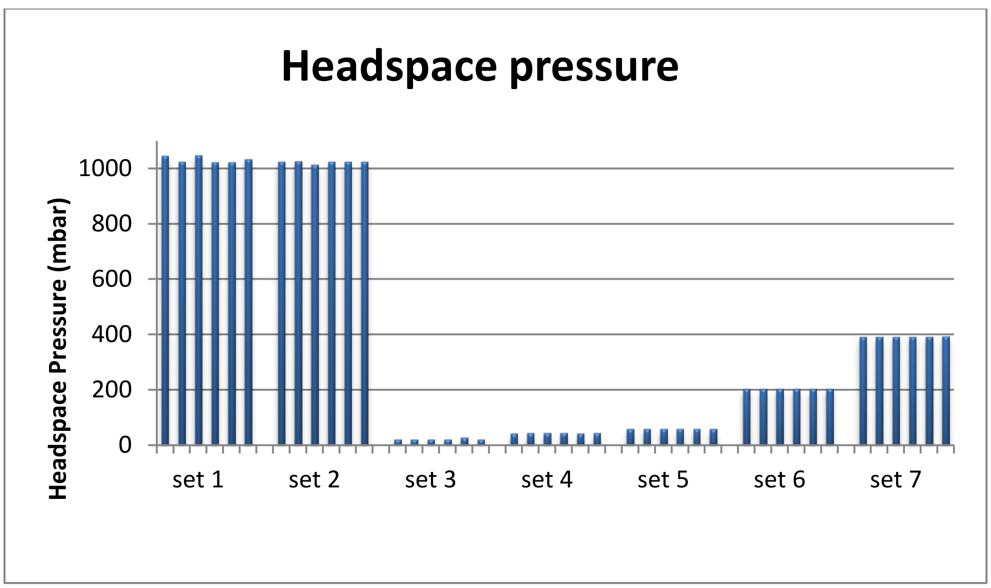


**Figure 5:** Measured headspace pressures in the 42 lyo product vials. The results are plotted in groups of six so that the seven sample sets are clearly visible.

### Headspace Oxygen Results

A set of known oxygen standards was measured to determine the performance of the headspace analyzer prior to sample measurement. The standards of known oxygen concentration were each measured three consecutive times. The mean measured headspace levels and the corresponding standard deviations are listed in Table 2.

**Table 2:** Mean measured headspace oxygen levels in known standards.

| Label | Headspace oxygen Mean (% atm) | Headspace oxygen St. Dev. (% atm) |
|---|---|---|
| 20% standard | 19.90 | 0.18 |
| 8% standard | 8.21 | 0.08 |
| 4% standard | 3.93 | 0.03 |
| 2% standard | 1.96 | 0.02 |
| 1% standard | 0.91 | 0.05 |
| 0% standard | 0.03 | 0.04 |

The measured headspace oxygen levels in the product vials are plotted in the Figure 6. The results are again plotted per set of six samples. The individual measurement results can be found in the appendix of this report.

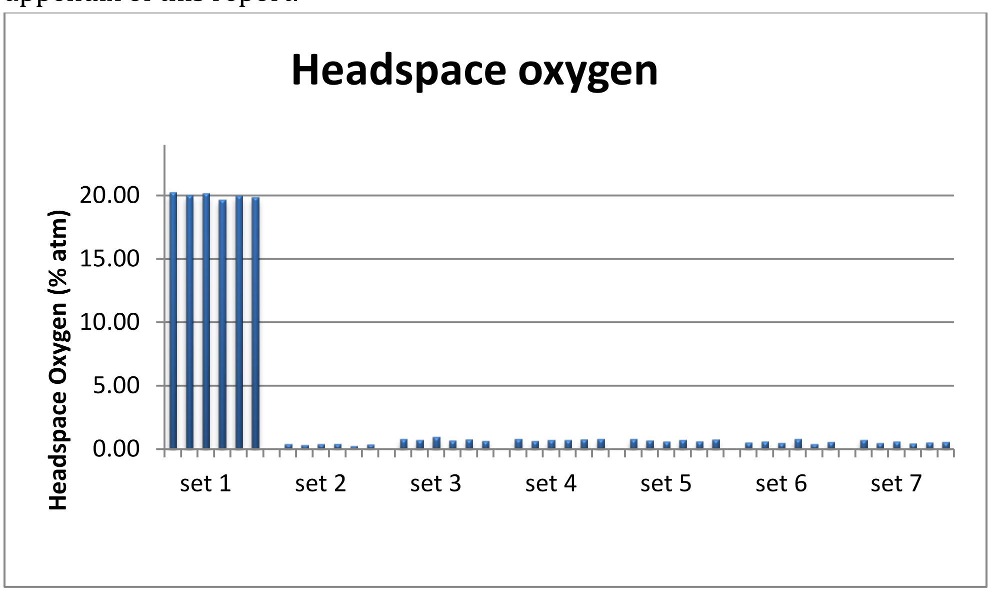


**Figure 6:** Measured headspace oxygen concentrations in the 42 lyo product vials. Note that the results are plotted in groups of six so that the seven sample sets are clearly visible.

**Headspace Moisture Results**
The measured headspace moisture levels in the provided product vials are listed in Figure 7. The individual measurements can again be found in the Appendix of this report.

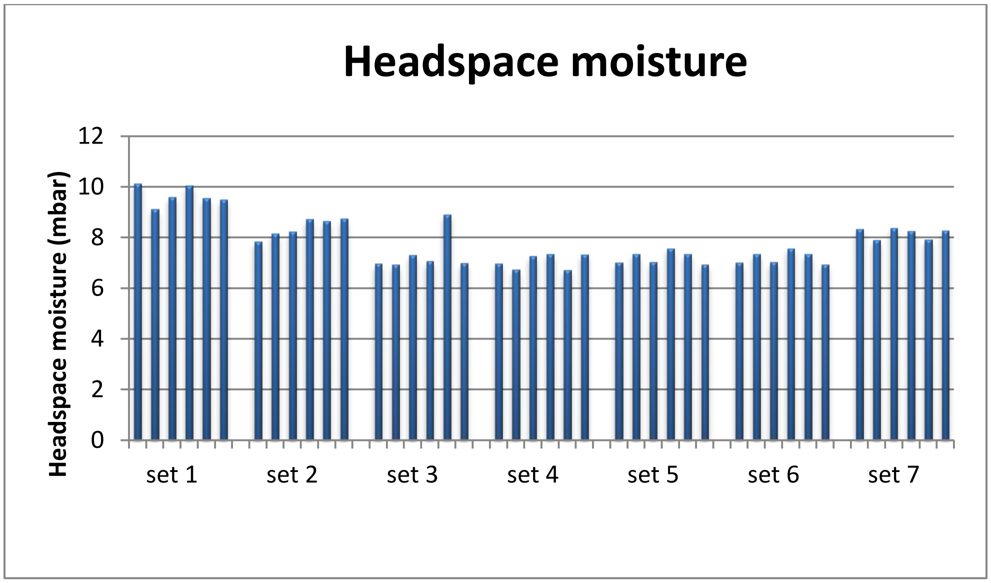


**Figure 7:** Measured headspace moisture levels in the 42 lyo product vials. Note that the results are plotted in groups of six so that the seven sample sets are clearly visible.

Without performing a correlation study, the measured headspace moisture levels provide information about the relative differences in total moisture content between the different samples. The headspace moisture levels can, in many cases, be correlated to the total moisture content obtained using Karl-Fisher titration. More information can be found in Reference 3 at the end of this report.

# Discussion

The results presented in the previous section show that headspace oxygen, pressure, and moisture measurements were possible with the provided product vials. These measurements together represent Total Headspace Characterization (THC). The THC concept measures all important headspace parameters in a lyo product sample, enabling product quality inspection and insight into the lyo process (see Reference [2] for an industry case study example).

Performing Total Headspace Characterization on the provided lyo vials enabled the following conclusions: As listed in Table 3, in the first sample set, samples are stoppered under atmospheric air. Sample set nr. 2 through sample set nr. 7 are samples stoppered under increasing partial pressures of nitrogen.

The headspace moisture ranges listed in Table 3 give insight into the efficiency of the drying process relatively between the different batches and into the homogeneity of drying within a batch (see References [3] and [4] for industry case study examples).

**Table 3:** Overview of the results of Total Headspace Characterization (THC) of the seven sets of lyo samples.

| Sample set nr. | Mean headspace pressure (mbar) | Mean headspace oxygen (% O2) | Headspace composition | Headspace moisture range (mbar) |
|---|---|---|---|---|
| 1 | 1032 | 19.98 | air | 9.13 – 10.13 |
| 2 | 1023 | 0.34 | nitrogen | 7.84 – 8.74 |
| 3 | 21 | 0.74 | nitrogen | 6.93 – 8.91 |
| 4 | 42 | 0.72 | nitrogen | 6.73 – 7.35 |
| 5 | 58 | 0.67 | nitrogen | 6.94 – 7.57 |
| 6 | 202 | 0.54 | nitrogen | 6.73 – 7.51 |
| 7 | 391 | 0.54 | nitrogen | 7.90 – 8.37 |

# Conclusions

Rapid non-destructive headspace inspection of 42 lyo product vials demonstrated that this method is suitable for performing container closure inspection. Both headspace oxygen and headspace pressure measurements demonstrated clear distinction of vials at varying levels of headspace pressure and headspace oxygen. It was also demonstrated that headspace moisture could be analyzed in the lyo product vials giving insight into the lyophilisation process itself.

The ability to rapidly and nondestructively measure headspace conditions in product samples enables insight into the process conditions that were used. In addition, this analysis can provide information about product quality. Applications for headspace inspection of lyophilized product are for example lyo cycle optimization, lyo chamber moisture mapping, container closure determination, and the monitoring stability vials over the shelf life.

## 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"
Downloadable at:

http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Whitepaper_CCI.pdf

2) LIGHTHOUSE Application Note 103: "Detecting Raised Stoppers in Sterile Freeze Dried Vials"
Downloadable at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Raised_Stopper_Detection_App_Note_103.pdf

3) LIGHTHOUSE White Paper: Using Laser-Based Headspace Moisture Analysis for Rapid Nondestructive Moisture Determination of Sterile Freeze-Dried Placebo
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_White_Paper_lyo_moisture.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/bookstore/PlaceboDetails.aspx?placeboabbreviation=17283

# Appendix

Listed below are the individual measurements for the 42 lyo product vials:

| SAMPLE SET 1 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 20.24 | 1045 | 10.13 |
| sample 2 | 20.01 | 1023 | 9.13 |
| sample 3 | 20.14 | 1047 | 9.61 |
| sample 4 | 19.68 | 1022 | 10.05 |
| sample 5 | 19.98 | 1022 | 9.55 |
| sample 6 | 19.84 | 1034 | 9.50 |

| SAMPLE SET 2 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 0.37 | 1024 | 7.84 |
| sample 2 | 0.30 | 1026 | 8.17 |
| sample 3 | 0.37 | 1013 | 8.23 |
| sample 4 | 0.39 | 1023 | 8.73 |
| sample 5 | 0.24 | 1024 | 8.66 |
| sample 6 | 0.34 | 1025 | 8.74 |

| SAMPLE SET 3 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 0.76 | 20 | 6.96 |
| sample 2 | 0.71 | 20 | 6.93 |
| sample 3 | 0.96 | 20 | 7.30 |
| sample 4 | 0.64 | 20 | 7.07 |
| sample 5 | 0.75 | 27 | 8.91 |
| sample 6 | 0.63 | 20 | 6.99 |

| SAMPLE SET 4 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 0.77 | 41 | 6.97 |
| sample 2 | 0.63 | 42 | 6.74 |
| sample 3 | 0.70 | 43 | 7.27 |
| sample 4 | 0.71 | 43 | 7.35 |
| sample 5 | 0.76 | 41 | 6.73 |
| sample 6 | 0.78 | 43 | 7.33 |

| SAMPLE SET 5 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 0.78 | 58 | 7.00 |
| sample 2 | 0.65 | 59 | 7.35 |
| sample 3 | 0.57 | 58 | 7.04 |
| sample 4 | 0.71 | 59 | 7.57 |
| sample 5 | 0.61 | 58 | 7.35 |
| sample 6 | 0.74 | 58 | 6.94 |

| SAMPLE SET 6 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 0.48 | 203 | 7.00 |
| sample 2 | 0.58 | 202 | 7.35 |
| sample 3 | 0.48 | 202 | 7.04 |
| sample 4 | 0.77 | 203 | 7.57 |
| sample 5 | 0.36 | 202 | 7.35 |
| sample 6 | 0.55 | 203 | 6.94 |

| SAMPLE SET 7 | | | |
|---|---|---|---|
| Label | Oxygen | Pressure | Moisture |
| sample 1 | 0.72 | 391 | 8.33 |
| sample 2 | 0.46 | 389 | 7.90 |
| sample 3 | 0.57 | 391 | 8.37 |
| sample 4 | 0.44 | 391 | 8.25 |
| sample 5 | 0.51 | 390 | 7.92 |
| sample 6 | 0.53 | 394 | 8.26 |