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Short communication

A novel GC–MS method for rapid determination of headspace oxygen in vials of pharmaceutical formulations

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ABSTRACT

A novel GC–MS method which requires small injection volumes was developed for fast and selective determination of headspace oxygen in pharmaceutical packages. This method does not require a specific GC column for separation of oxygen from other permanent gases such as nitrogen; instead it offers the advantage of using co-eluting nitrogen as the internal standard for quantifying oxygen in the headspace under electron ionization (EI, 70 eV) conditions. The relative ionization efficiency of oxygen to nitrogen, termed as ionization efficiency correction factor (IECF), can be measured using a control sample with known composition of oxygen and nitrogen such as the standard dry air used in this study. To avoid contamination, it is necessary to flush the syringe with pure helium. The measurements by the method are independent of the variations of sampling volumes. The determined headspace oxygen contents (R.S.D. < 1%) in the containers of an investigational intravenous formulation using this method are consistent with the results obtained by an oxygen instrument at the manufacturing facility. The performance of the analytical approach was evaluated in the study of the container closure integrity at various storage conditions including upright and inverted orientations. The results suggest that there is no obvious oxygen penetration over 12 months. This method provides a convenient tool for measuring the levels of HS oxygen in vials of pharmaceutical formulations.

1. Introduction

Although oxidative degradation of drug substances in pharmaceutical formulations is well known [1,2], the mechanism to promote reactions between active pharmaceutical ingredients and molecular oxygen in pharmaceutical formulations could be complicated. For example, some degradation reactions were proposed via radical auto-oxidation processes [2]; while the others underwent [4+2] Diels-Alder cycloaddition reaction with singlet oxygen upon photo-irradiation [3]. As a result, protection from oxygen is necessary to prolong the shelf life of pharmaceutical formulations [1]. The effectiveness of nitrogen purging/flushing and the integrity of container closure systems have significant impacts on the product stability (shelf life). Since the headspace (HS) oxygen content is a good measure for both, fast determination of HS oxygen can guide the development and implementation of pharmaceutical packaging procedures.

Several types of oxygen analysis techniques including headspace gas chromatography (HS-GC), electrochemical methods, frequency modulation spectroscopy, and fluorescence-quenching methods have been reported [4]. Mass spectrometric analysis of permanent gases using a modified ion trap was specifically developed for monitoring cryogenic fuel leaks within a Space Shuttle; however direct analysis without instrumentation modification was not reported in this study [5]. To the authors' knowledge, fast and accurate determination of HS oxygen remains challenging for pharmaceutical packaging applications, in terms of sampling and performing accurate oxygen measurements of very small headspace volumes (e.g. <1000 μL). This is especially true when an existing instrument such as GC–MS is available in a typical spectroscopy lab.

Recently, micro-gas chromatography (μGC) method was developed for rapid analysis of headspace oxygen and moisture simultaneously on sample volumes of 50–100 μL by employing μGC with dual chromatographic analysis modules [6]. Using microscale instrumentation, μGC was demonstrated to be able to achieve oxygen separation from argon and nitrogen in less than 90 s, compared to relatively lengthy separation by a conventional GC (~8 min). However, the method using GC (including μGC) requires separation of oxygen from other permanent gases (such as nitrogen and argon, etc.) using a porous-layer (molecular sieve) open tubular (PLOT) column followed by thermal conductivity detection (TCD). Although separation of oxygen from nitrogen and argon can be

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achieved by careful optimization of experimental conditions, TCD is a non-selective detector and hence reference standards are required for peak identification. In order to obtain a specific detection, mass spectrometric detection would be ideal. However, a PLOT column cannot be readily connected to a mass spectrometer directly due to difficulty in interfacing high volume of carrier gas to vacuum.

Currently, an investigational intravenous formulation is under development for the treatment of cancer. However, the drug substance used to make this formulation is sensitive to oxygen and product stability is of concern. Thus, packaging procedures to reduce HS oxygen must be implemented during manufacturing process to ensure product stability. A method for rapid measurement of HS oxygen contents is of great importance for evaluation of the container closure integrity, establishing stability and HS oxygen relationship during transportation and storage.

Here, we report a novel and fast GC-EI/MS method developed for analysis of HS oxygen in closed containers. Our studies focused on (1) development and optimization of the analytical method using an existing conventional GC-EI/MS instrumentation with a common capillary column and (2) demonstration of determination of the HS oxygen contents in the I.V. formulation containers in various storage conditions.

2. Experimental

The I.V. formulation vials were prepared at the oncology manufacturing facility using an automated nitrogen flushing machine and were followed by the detection using an Obisphere 510 oxygen instrument equipped with an electro chemical sensor (Hach Ultra Analytics, Geneva, Switzerland). A Hamilton 1802 RN gastight syringe (25 μL, Hamilton Company, Reno, NV, USA) was used to circumvent injection contamination from oxygen in the atmospheric air. The syringe needle was flushed three times with helium (4.6 Zero Grade, 99.996%) (Praxair, Danbury, CT, USA) before sampling HS oxygen in the closed formulation vials.

GC-EI/MS analysis was performed on a PolarisQ coupled to a Trace GC (Thermo Electron, Tampa, FL) equipped with an EI source. Helium (4.6 Zero Grade, 99.996%) (Praxair, Danbury, CT, USA), was used as the carrier gas. Medical grade dry air (Praxair, Danbury, CT, USA) was used as the standard to calibrate the EI/MS intensity ratio of oxygen to nitrogen. A conventional GC fused silica column DB-5MS (30 m × 0.25 mm, 0.25 μm) (J&W Scientific, CA, USA) was utilized to obtain the retention of nitrogen and oxygen. Note that other weak polar columns such as DB-624 (30 m × 0.25 mm, 0.25 μm) (J&W Scientific, CA, USA) could give similar results. The GC oven temperature was initially held for 1 min at 50 °C and then ramped up to 100 °C in 1 min. Total run time is 2 min. The constant flow rate of the carrier gas was 1.5 mL/min. GC–MS data were recorded and analyzed using Xcalibur Version 1.4 software. All measurements were averages of triplicate runs to statistically increase accuracy.

3. Results and discussion

3.1. GC-EI/MS method development

Owing to the specificity of MS detection, there is no need to separate oxygen from nitrogen for analysis. Instead, we took the advantage of co-elution of O_2 and N_2 so that N_2 was used as the internal standard for quantifying HS oxygen in the closed containers. Under electron ionization (EI, 70 eV) conditions, the co-eluting oxygen and nitrogen can be ionized to radical cations (Eqs. (1) and (2)).

$$O_2 + e^- * \rightarrow {O_2}^{+\bullet} + 2e^- \quad (1)$$

$$N_2 + e^- * \rightarrow {N_2}^{+\bullet} + 2e^- \quad (2)$$

The ionization efficiency of O_2 differs from that of N_2. This ionization efficiency difference, however, can be corrected using a control sample (standard dry air with 20.95% oxygen and 79.02% nitrogen in this case) based on the MS intensity ratio of oxygen to nitrogen ($I_{(\mathrm{Air-O_2})}/I_{(\mathrm{Air-N_2})}$). The ionization efficiency correction factor (IECF) can be derived from Eq. (3).

$$\mathrm{IECF} = \frac{I_{(\mathrm{Air-O_2})}/I_{(\mathrm{Air-N_2})}}{20.95\%/79.02\%} = 3.772 \left(\frac{I_{(\mathrm{Air-O_2})}}{I_{(\mathrm{Air-N_2})}}\right) \quad (3)$$

For real sample analysis of HS oxygen, the measured percentage of oxygen can be obtained from Eq. (4).

$$\%(\mathrm{O_2}) = \frac{(I_{(\mathrm{HS-O_2})}/I_{(\mathrm{HS-N_2})})/\mathrm{IECF}}{\{(I_{(\mathrm{HS-O_2})}/I_{(\mathrm{HS-N_2})})/\mathrm{IECF}\} + 1} \times 100 \quad (4)$$

There are two approaches to obtain the intensity ratio of oxygen to nitrogen. One is based on the peak areas which were integrated by applying the Trapezoidal Rule [7] multiple times across a peak. It was done at regular time increments and subsequently summed the individual trapezoid area as shown in Eq. (5), where $\mathrm{height}_{\mathrm{start\ time}}$ and $\mathrm{height}_{\mathrm{end\ time}}$ are the peak heights at the first and last increments, respectively.

$$\mathrm{Area} \cong \frac{\Delta t}{2}\left(\mathrm{height}_{\mathrm{start\ time}} + 2\sum_{i=2}^{n-1}\mathrm{height}_i + \mathrm{height}_{\mathrm{end\ time}}\right) \quad (5)$$

The number of time increments, which is the same as the number of scans across a peak, is given by n. Δt, which represents the time increment of each trapezoid, is calculated for each scan from Eq. (6).

$$\Delta t = \frac{\mathrm{end\ time} - \mathrm{start\ time}}{n-1} \quad (6)$$

The second approach is to use the peak intensity, provided that the oxygen and nitrogen completely co-elute as in our current study. By this approach, one can simply use peak heights to calculate the intensity ratio of oxygen peak at m/z 32 to nitrogen peak at m/z 28 in an average MS spectrum over n, the number of scans across a peak.

3.2. Optimization of GC conditions

Typical total ion chromatograms (TICs) and extracted ion chromatograms (EICs) of the blank (a run without injection), the standard dry air, and the HS of a closed vial are displayed in Fig. 1. It shows that oxygen and nitrogen co-elute at 1.2 min using a J&W DB-5MS column.

Although the initial GC oven temperature can be set up to as low as 30 °C (due to the fact that oxygen and nitrogen are gases), the peak shape and resolution did not exhibit obvious difference at initial oven temperature 30, 40, and 50 °C, respectively. Therefore, the initial GC oven temperature was set to 50 °C to avoid longer equilibrium time. In addition, the oven temperature was ramped to 100 °C to help remove trace moisture from the GC column.

The effects of different injection volumes (10, 25, and 50 μL) on the method were also evaluated. Fig. 2 displays the measurements for oxygen and nitrogen by integration of peak areas using Eq. (5), and the intensity ratio is 0.1368 for this particular example. An example of the averaged MS spectra of air and HS are shown in Fig. 3, from which the peak heights of oxygen and nitrogen were obtained to calculate the intensity ratios ($I_{(\mathrm{HS-O_2})}/I_{(\mathrm{HS-N_2})}$). Based on the measured MS intensity ratios using peak areas and peak heights as well as the theoretical percentage ratio of oxygen to

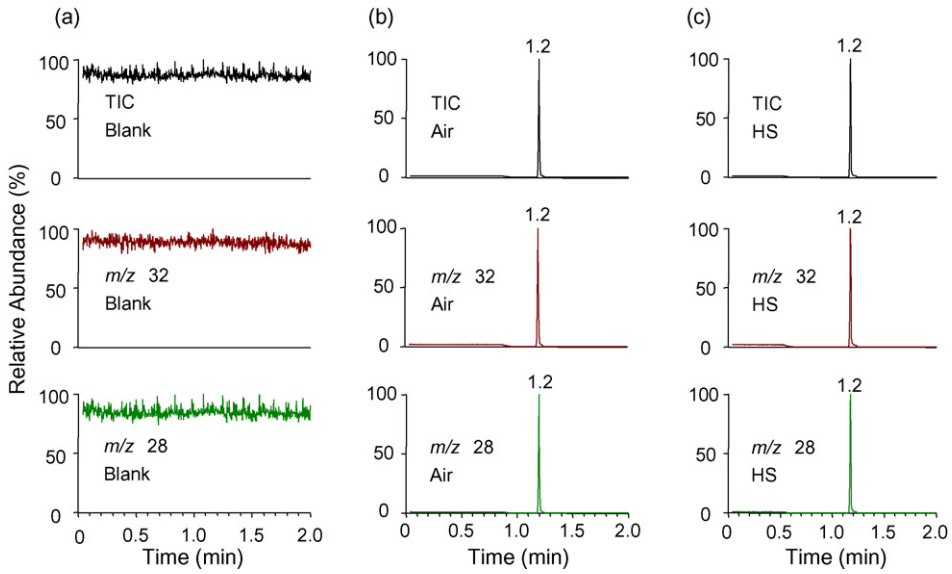

Fig. 1. Typical total ion chromatograms (top panel), extracted ion m/z 32 of O_2 (middle panel), m/z 28 of N_2 (bottom panel) in blank (a), control air (b), and HS of a closed vial (c), respectively.

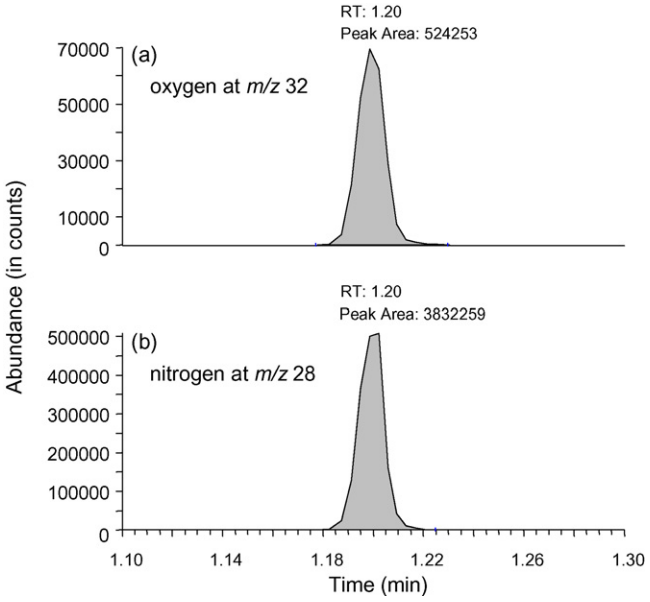

Fig. 2. Peak area integrations of HS oxygen and nitrogen in a typical MS measurement based on Eq. (5).

nitrogen in the control sample (the standard dry air), IECFs corresponding to each injection volume (e.g. 10, 25, and 50 μL) were determined from Eq. (3) and summarized in Table 1. These relative factors were used to calculate the percentages of oxygen in the HS of the containers based on Eq. (4). The results for the analysis of HS oxygen of a typical sample 367 were tabulated in Table 1, from which the effects of injection volumes on the measurements could be compared. For example, when peak areas were used for calculation of $I_{(O_2)}/I_{(N_2)}$, the measured percentages of HS oxygen are 13.79, 13.81, and 13.67 (all with less than 0.8% R.S.D.) at the injection volumes of 10, 25, and 50 μL, respectively. As for the use of peak heights, similar results (within 0.8% R.S.D.) were obtained. This indicates that the measurements are independent of the variations of sampling volumes. Accordingly, the injection volume of 10 μL was chosen for measuring HS oxygen of small headspace volumes (e.g. less than 1000 μL) in the following analysis.

3.3. Analysis of the HS oxygen

In order to test the measurement consistency of this method with other oxygen analysis technique, on-line detection using an Obisphere 510 oxygen instrument was performed at the manufacturing facility. The measured percentages of HS oxygen on two representative vials prepared from the beginning and the end of

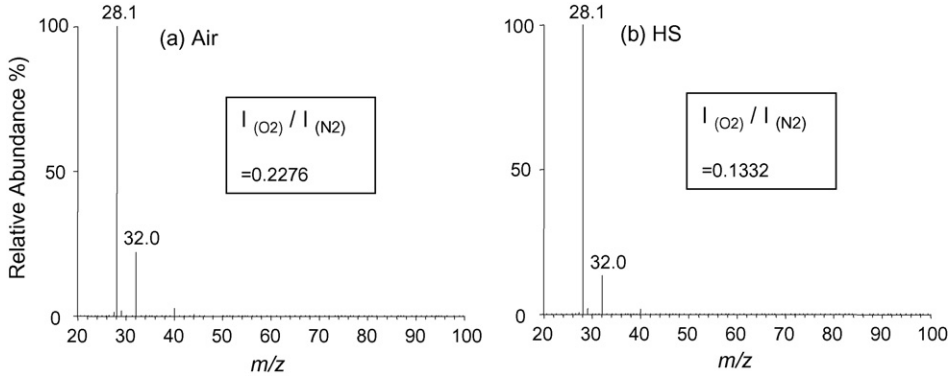

Fig. 3. Intensity ratios of oxygen to nitrogen in averaged MS spectra of the air and HS samples.

Table 1
Effects of injection volumes on determination of HS oxygen of a typical sample 367 based on the measured intensity ratios of oxygen to nitrogen

Sample	Injection volume (μL)	$I_{(O_2)}/I_{(N_2)}$			Average	R.S.D. (%)	IECF	Measured % oxygen[a]
		Run #1	Run #2	Run #3				
Air[b]	10	0.2239[c]	0.2248[c]	0.2256[c]	0.2248	0.39		–
Air[b]	10	0.2276[d]	0.2303[d]	0.2273[d]	0.2284	0.72		–
367	10	0.1349[c]	0.1369[c]	0.1353[c]	0.1357	0.78	0.8478	13.79
367	10	0.1332[d]	0.1333[d]	0.1348[d]	0.1338	0.66	0.8615	13.44
Air[b]	25	0.2239[c]	0.2248[c]	0.2256[c]	0.2245	0.45		–
Air[b]	25	0.2276[d]	0.2303[d]	0.2273[d]	0.2297	0.65		–
367	25	0.1349[c]	0.1369[c]	0.1353[c]	0.1357	0.74	0.8469	13.81
367	25	0.1332[d]	0.1333[d]	0.1348[d]	0.1337	0.73	0.8666	13.37
Air[b]	50	0.2239[c]	0.2248[c]	0.2256[c]	0.2264	0.40		–
Air[b]	50	0.2276[d]	0.2303[d]	0.2273[d]	0.2269	0.69		–
367	50	0.1349[c]	0.1369[c]	0.1353[c]	0.1352	0.67	0.8540	13.67
367	50	0.1332[d]	0.1333[d]	0.1348[d]	0.1347	0.74	0.8559	13.60

[a] Measured % oxygen is based on Eqs. (3) and (4).
[b] Standard dry air which contains N_2: 79.02%, O_2: 20.95%.
[c] The measurement of $I_{(O_2)}/I_{(N_2)}$ was based on peak areas of oxygen and nitrogen using Eq. (5).
[d] The measurement of $I_{(O_2)}/I_{(N_2)}$ was based on peak heights in an average MS spectrum.

the filings are 9.73% and 8.81%, respectively, giving an average of 9.27%. By using GC–MS method, the measured HS oxygen levels of two representative vials taken from the beginning and the end of the filings are 9.39% and 9.28%, yielding an average of 9.34%. For this particular example, the measurement difference is 0.07% using the oxygen instrument and GC–MS method. Note that these samples mentioned above were prepared using improved nitrogen flushing procedures and hence exhibited lower HS oxygen levels than the samples discussed below.

The determination of the HS oxygen contents in the formulation containers in various storage conditions including upright and inverted orientations were performed at optimized GC conditions and an injection volume of 10 μL. Note that IECFs obtained at the injection volume of 10 μL (Table 1) were applied to the determination of oxygen levels in the HS of the formulation containers (Table 2). By comparison with the measured HS oxygen levels at the beginning of storage (data not shown), the measured HS oxygen contents after storage for 12 months suggest that there were no significant oxygen penetration irrespective of the storage conditions. It appears that there are some discrepancy (from 0.06% for sample 529 to 0.35% for sample 367) between the measurements of oxygen percentages using the peak areas by integration and the peak heights from an averaged MS spectrum. This probably resulted from the variations between the Trapezoidal Rule for peak integration and algorithm parameters used converting the TIC of a peak to obtain peak intensities in an averaged MS spectrum; but detailed discussion is beyond the scope of this short communication.

The calculated R.S.D. in Table 2 are in less than 1% for all analysis, partially resulting from intrinsic characteristics of the method in that the variation in sampling volume does not affect the measurements. However, there are some variations observed for the vials at the identical conditions. For example at the conditions of ambient with inverted storage, the intensity ratios for the samples 155 and 167 are 13.10% and 13.65%, respectively, giving about 0.55% difference between these two samples. This is the largest variation among all sample pairs stored at the identical conditions (e.g. 5 °C-50%RH/upright, 5 °C-50%RH/inverted, 25 °C-60%RH/upright, 25 °C-60%RH/inverted). This difference probably

Table 2
Summary of determined percentages of oxygen in HS of the formulation containers in various storage orientations

Sample	Storage condition	$I_{(O_2)}/I_{(N_2)}$			Average	R.S.D. (%)	Measured % oxygen[a]
		Run #1	Run #2	Run #3			
Air[b]	Ambient	0.2239[c]	0.2248[c]	0.2256[c]	0.2248	0.39	–
Air[b]	Ambient	0.2276[d]	0.2303[d]	0.2273[d]	0.2284	0.72	–
367	5 °C-50%RH/upright[e]	0.1349[c]	0.1369[c]	0.1353[c]	0.1357	0.78	13.79
367	5 °C-50%RH/upright[e]	0.1332[d]	0.1333[d]	0.1348[d]	0.1338	0.66	13.44
529	5 °C-50%RH/upright[e]	0.1379[c]	0.1375[c]	0.1372[c]	0.1375	0.25	13.96
529	5 °C-50%RH/upright[e]	0.1388[d]	0.1383[d]	0.1402[d]	0.1391	0.69	13.90
155	5 °C-50%RH/inverted[e]	0.1304[c]	0.1293[c]	0.1313[c]	0.1303	0.76	13.32
155	5 °C-50%RH/inverted[e]	0.1305[d]	0.1291[d]	0.1302[d]	0.1299	0.55	13.10
167	5 °C-50%RH/inverted[e]	0.1355[c]	0.1364[c]	0.1362[c]	0.1360	0.32	13.82
167	5 °C-50%RH/inverted[e]	0.1374[d]	0.1358[d]	0.1355[d]	0.1362	0.76	13.65
798	25 °C-60%RH/upright[e]	0.1361[c]	0.1364[c]	0.1369[c]	0.1364	0.32	13.84
798	25 °C-60%RH/upright[e]	0.1337[d]	0.1357[d]	0.1358[d]	0.1351	0.88	13.55
901	25 °C-60%RH/upright[e]	0.1355[c]	0.1350[c]	0.1347[c]	0.1351	0.29	13.74
901	25 °C-60%RH/upright[e]	0.1346[d]	0.1338[d]	0.1345[d]	0.1343	0.32	13.49
561	25 °C-60%RH/inverted[e]	0.1364[c]	0.1377[c]	0.1355[c]	0.1365	0.78	13.87
561	25 °C-60%RH/inverted[e]	0.1371[d]	0.1360[d]	0.1361[d]	0.1364	0.45	13.67
612	25 °C-60%RH/inverted[e]	0.1353[c]	0.1354[c]	0.1368[c]	0.1358	0.61	13.81
612	25 °C-60%RH/inverted[e]	0.1336[d]	0.1329[d]	0.1323[d]	0.1329	0.48	13.37

[a] Measured % oxygen is based on Eqs. (3) and (4).
[b] Standard dry air which contains N_2: 79.02%, O_2: 20.95%.
[c] The measurement of $I_{(O_2)}/I_{(N_2)}$ was based on peak areas of oxygen and nitrogen using Eq. (5).
[d] The measurement of $I_{(O_2)}/I_{(N_2)}$ was based on peak heights in an average MS spectrum.
[e] RH represents relative humidity.

indicates the variation during nitrogen flushing at the manufacturing facility.

4. Conclusions

A novel GC-EI/MS method was developed to successfully analyze the oxygen contents in the HS of the I.V. formulation containers. The method is simple, rapid and convenient and requires very small injection volume (10 μL is sufficient in comparison with the requirement of at least 2 mL sample volume using the oxygen instrument at the manufacture facility) due to the direct use of a conventional GC–MS set up with a generic column such as J&W DB-5MS. In order to overcome contamination from atmosphere, flushing the gas-tight syringe with pure helium before a measurement is necessary. It is predicted that large injection volume can minimize the effect due to oxygen contamination from the atmospheric pressure, especially when the oxygen percentage in HS is very small (e.g. <2%). Thus, future work will focus on improving the method for analysis of HS samples at low oxygen percentages and automation.

This study suggests that the integrity of container closure remains good (no oxygen penetration) for 12 months at various storage conditions including upright and inverted orientations. The results of HS oxygen levels help define manufacturing process parameters to ensure the product stability. The method offers a useful tool for the ongoing kinetic study by establishing the relationship between the quantities of degradation impurities and HS oxygen levels.

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