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Evaluation of propylene glycol methyl ether as a potential challenge agent for leak detection of liquid and headspace from closed system drug transfer devices using Fourier transform infrared spectroscopy

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Abstract

Choosing an appropriate surrogate of hazardous drugs for use in testing Closed System Drug-Transfer Devices (CSTDs) is a challenging endeavor with many factors that must be considered. It was suggested that the compound propylene glycol methyl ether (PGME) may meet many of the criteria we considered important in a suitable surrogate. Criteria included sufficient volatility to evaporate from aqueous liquid leaks efficiently, a Henry's constant which produced sufficient vapor phase concentrations to make headspace leaks detectable, and suitability for detection using a low-cost detection system. We evaluated the measurement of vapors from solutions containing PGME released inside a closed chamber. We present data used to quantify limits of detection, limits of quantification, bias, precision, and accuracy of Fourier Transform Infrared Spectroscopy (FTIR) measurements of vapors from 2.5 M PGME solutions. The effects of ethanol as a component of the PGME solution were also evaluated. Liquid drops of PGME solutions and headspace vapors above PGME solutions were released to simulate leaks from CSTDs. Using a calibration apparatus, an instrumental limit of detection (LOD) of 0.25 ppmv and a limit of quantitation (LOQ) of 0.8 ppmv were determined for PGME vapor. A LOD of 1.1 μL and a LOQ of 3.5 μL were determined for liquid aliquots of 2.5 M PGME solution released in a closed chamber. Accurate quantitation of liquid leaks required complete evaporation of droplets. With the upper end of the useable quantitation range limited by slow evaporation of relatively large droplets

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Author contributions

Emily G. Westbrook's contributions to the work herein include investigation, data curation, formal analysis, visualization and writing of the original draft of this work. Amos Doepke contributed to the conceptualization, methodology, project administration, procurement of resources, supervision, visualization, writing and reviewing of this work. Robert P. Streicher contributed to the conceptualization, methodology, project administration, supervision, writing and reviewing of this work.

Conflicts of interest

There are no conflicts to declare.

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and the lower end defined by the method LOQ, the method evaluated in this research had a narrow quantitative range for liquid droplets. Displacement of 45 mL of vial headspace containing PGME vapor is the largest amount expected when using the draft NIOSH testing protocol. Release of an unfiltered 45 mL headspace aliquot within the NIOSH chamber was calculated to produce a concentration of 0.8 ppmv based on the Henry's constant, which is right at the instrumental LOQ. Therefore, the sensitivity of the method was not adequate to determine leaks of PGME vapor from a headspace release through an air filtering CSTD when using the draft NIOSH testing protocols with an FTIR analyzer.

1. Introduction

The work herein was conducted to investigate the utility of 2.5 M solutions of propylene glycol methyl ether (PGME) as a surrogate for hazardous drugs (HDs) when leak testing of Closed System Drug-Transfer Devices (CSTDs). Without actual testing of CSTDs, we present a comprehensive set of experiments and analysis of a HD surrogate, exploring the evaporation of PGME solutions and the detectability of PGME vapors by FTIR in the NIOSH chamber. We have chosen to work in this system, and investigate use of PMGE, as a modification of previous work undertaken by NIOSH researchers to develop a CSTD testing protocol.[1,2] When a surrogate compound is used, the properties of the compound and the sensitivity of the detection system combine to set the limits of detection and quantitation, which in turn limits the leak sizes that can be determined. The detectability of any proposed surrogate must be rigorously evaluated. The draft protocol presented in Hirst *et al.*,[1] proposed detection of isopropanol (IPA) from a 70% IPA solution, in a chamber *via* IR detection. Work by Szkiladz and Hegner demonstrate some of the limitations of the Hirst *et al.* protocol, such as an inability to quantify leak volumes and differentiate performance of the CSTDs.[1,3] In 2016, NIOSH expressed a desire to extend the usability of the 2015 draft protocol to include air filtering type CSTDs.[1,2] In the work herein, we have adapted the chamber from the draft protocol in Hirst *et al.*[1] and use a more sensitive FTIR detector to decrease limits of quantification. Compared to analysis techniques such as GC-MS, the FTIR gas analysis allows for near real-time leak detection, which may be beneficial for identifying processes and techniques where CSTDs are leaking. Additionally, we explore methods for evaluating the performance of a surrogate solution and make calibrations of generated leak volumes *versus* observed vapor concentrations.

Reports of CSTD testing in the literature use a variety of methods to challenge the integrity of a CSTD system against the escape of hazardous drug (HD) liquid or vapor concentrations outside of the system that may potentially cause an exposure to the health care worker.[4–9] Studies in the literature that evaluate leak testing for quantifying CSTD efficacy, often use a surrogate compound as the detected chemical in place of the HD.[10–12] Use of a surrogate can increase the sensitivity of a test compared to using low volatility HDs, which can be difficult to detect in manipulations of CSTDs intended to simulate real world use scenarios, such as when transferring drugs from a vial.

However, when substituting an HD for a surrogate, in an aqueous environment, vapor pressure and Henry's constant become significant variables.[10,13] A variation of the NIOSH

draft protocol[1] was presented in Wilkinson *et al.*,[12] that used FTIR gas-phase detection of 2-phenoxyethanol (2-POE) from aqueous solutions as a method to detect leaks from CSTDs. Previous work using semi-volatile compounds as leak detection markers suffered from slow evaporation and adsorption losses which complicated or prevented correlating leak volumes with gas phase measurements.[10] In our own evaluation of 2-POE, the method seemed to suffer significant analytical challenges due to the low volatility of 2-POE (2020 research by Westbrook; unreferenced†). Wilkinson suggested that the use of PGME, which is a much more volatile compound than 2-POE, may be an effective indicator of leaks (2020 data presented by Wilkinson to NIOSH; unreferenced‡). In order to develop an acceptable testing protocol, it seemed incumbent to evaluate PGME. It was theorized that the relatively volatile PGME may allow complete evaporation and subsequent measurement of PGME vapors in a time frame conducive to testing and determining leak volumes. We evaluated a solution of 2.5 M PGME in water with and without the presence of 30% ethanol. Ethanol is used in some HD formulations as a diluent, which would necessitate inclusion of ethanol in a comprehensive testing protocol.[14–16] From the experimental investigations, we have determined several strengths and limitations of the method using FTIR to measure PGME vapor as an indicator of a leak.

2. Materials and methods

2.1 Materials

Ethanol and 1-methoxypropan-2-ol (PGME) were from the Sigma-Aldrich Co (Saint Louis, MO). Water was deionized to >18 MΩ cm using an Evoqua Water Technologies (Pittsburgh, PA) water purification system. Gasmet DX4040 FTIR Gas Analyzer and Calcmet software were from Gasmet Technologies Oy (Vantaa, Finland). Adsorbent Tube Injector System (ATIS) was from Supelco Inc. (Bellefonte, PA). Syringe pumps were from Cole-Parmer (Vernon Hills, IL). Gas-tight syringes were from Hamilton Company (Reno, NV). A Bios DryCal Defender flow-meter was from Mesa Labs (Lakewood, CO).

A Secador® Techni-dome® 360 Large Vacuum Desiccator from Bel-Art Products (Pequannock, NJ) was customized by adding a 30 cm tall cylinder the same diameter as the desiccator between the desiccator halves. Glove ports (20 cm dia.) in the cylinder enabled the desiccator to be used as a glove chamber with a volume of 131 liters. A fan was used to circulate air within the chamber. The configuration of the chamber including the cylinder with glove ports is referred to as the NIOSH chamber.

2.2 Calibration of the FTIR to PGME

The Gasmet DX4040 FTIR Gas Analyzer is an infrared spectrometer, with an 8 cm^{-1} resolution in the range from 900 to 4200 cm^{-1}. The DX4040 is equipped with a temperature-controlled sample cell of 0.4 L, and a 9.8 m effective path length. An apparatus was assembled to calibrate the FTIR spectrometer by quantitatively introducing neat liquid PGME and/or ethanol into a flow of nitrogen gas while FTIR spectra were recorded as

†E. G. Westbrook, NIOSH research done to evaluate the use of 2-POE as a surrogate, Presented internally, Unpublished, March 2020.
‡A.-S. Wilkinson, Biopharma Stability Testing Laboratory, Presentation of data to NIOSH regarding the detectability of PGME, Unpublished, March 2020.

depicted in Fig. 1. Two syringe pumps were fitted with glass syringes, with one containing PGME and the other containing ethanol. The syringes were connected to PEEK tubing which was inserted through the septum of the ATIS. Bottled nitrogen gas, controlled by a regulator on the N_2 tank and a needle valve on a ball flow meter, was connected to the inlet of the ATIS. The ATIS temperature was set to 50 °C. The flow rate of gas exiting the ATIS was set to 2.40 L min^{-1} as measured using a calibrated Bios DryCal flowmeter. The rates of the syringe pumps were adjusted to achieve the desired final concentration of PGME and/or ethanol when mixed with the nitrogen flow. The effluent from the ATIS was connected to the sample inlet port of the FTIR spectrometer. The FTIR spectrometer was operated with a cell temperature of 30 °C. FTIR measurements in the calibration apparatus used a background spectrum of N_2 gas. The recorded spectra were converted to a calibration library for automated instrumental determination of PGME concentrations.

2.3 Gravimetric analysis of the evaporation of 2.5 M aqueous PGME

Estimates of the evaporation times for various volumes of PGME solution were made by gravimetric analysis and observation. All gravimetric measurements were done at room temperature using a Mettler Toledo (Columbus, OH), model XP205DR analytical balance. A single drop of PGME solution was placed on a tared, 75 mm dia. borosilicate watch glass, in the analytical balance. With the balance draft-shield doors open, a fan was used to move air over the solution to aid evaporation and simulate conditions used in later experiments inside the NIOSH chamber. The draft shield doors were closed at one-minute intervals and the mass was recorded. The time for complete evaporation was confirmed by visual observations indicating that the solution aliquot was gone from the watch glass.

2.4 Measurements of PGME vapor in the NIOSH chamber

A port on the upper section of the NIOSH chamber was connected to the FTIR sample inlet *via* $\frac{1}{4}$ inch PTFE tubing. The FTIR instrument had a sample flow rate of 2.5 liters per minute. FTIR is not a destructive analysis method, so to reduce the loss of atmosphere due to sampling, the exhaust of the FTIR was returned to the chamber as shown in Fig. 2.

A fan (3.5 inch dia.) was used to circulate the air inside the NIOSH chamber to promote evaporation of the PGME solutions and homogeneity of the atmosphere. When measuring PGME in the chamber, FTIR background spectra were recorded in lab air (at room temperature), so that the PGME signal was discernable without needing to subtract water and other background components as part of the post collection analysis of the spectrum. FTIR measurements of PGME vapor from a liquid source inside the NIOSH chamber were done after dispensing aliquots of 2.5 M PGME from syringes onto a watch glass where they could evaporate.

Headspace from above a 2.5 M PGME solution in a vial was measured in the NIOSH chamber. Measurements were made of PGME vapor generated from the headspace using a 100 mL drug vial with a crimp-cap septum containing 50 mL of 2.5 M PGME solution. A needle, on a 50 mL syringe filled with lab air was inserted through the septum of the drug vial. A second needle was inserted into the septum to provide a path for headspace effluent. From the syringe, 50 mL of air was pushed into the drug vial, which forced headspace from

the vial *via* the second needle. After pushing the air from the syringe, both needles were removed from the septum during the measurement period.

3. Results and discussion

3.1 FTIR absorbance spectra for a range of PGME concentrations

The FTIR gas analyzer was designed for measurement of compounds at low concentrations in ambient air. PGME vapors with concentrations calculated from syringe flow rate and nitrogen flow rate were generated using the calibration apparatus described above. Increasing the ATIS temp, from room temperature (~22 °C) up to 95 °C had no effect on the measured PGME concentration of a theoretical 25 ppmv PGME gas sample, which was inferred to mean complete evaporation of the PGME was occurring in the ATIS at 22 °C. The ATIS temperature was set to 50 °C for all other experiments.

The Gasmet FTIR has accompanying Calcmet software which was used to program the FTIR and report the concentrations of analytes. Using the linear regression calculated from the reference library spectra, the instrument output reports a vapor concentration from the observed spectrum. The Calcmet software automatically fits reference spectra to the observed spectrum and calculates areas under the curve in the analysis regions. The areas are fit to calibration curves generated from a library of reference spectra to calculate an analyte concentration. For the analytes of interest, the Calcmet software outputs a concentration. The residual, after subtracting reference spectra of the compounds from the sample spectrum, is also reported by the Calcmet software as an indicator of the accuracy of the identification and quantification.

FTIR spectra were collected (Fig. 3) with a 5 second integration time and one second intervals, totaling a six second interval for measurements. The spectra agree well with NIST standard reference data for infrared spectra of the compounds PGME and ethanol.[17,18] PGME was quantified in the following wavenumber regions, 800 to 1600, 2500 to 3300, and 3500 to 3800 cm^{-1}. Ethanol was quantified in the following wavenumber regions, 800 to 1700, 2550 to 3200, 3500 to 3800 cm^{-1}.

3.2 Calibration and characterization of instrument response to PGME in nitrogen

Two syringe pumps were used, one with PGME and the other with ethanol, to create known PGME and ethanol concentrations using the calibration apparatus. Calculated PGME concentrations of 125, 100, 50, 25, 10, and 5 ppmv samples generated in the calibration apparatus produced a steady-state instrument response. The spectral response to ethanol was recorded for theoretical concentrations of 100, 75, 50, 25, and 10 ppmv ethanol. The spectra from PGME and ethanol were recorded and entered into the instrument library as reference spectra. A linear calibration curve of the absorbance area, for specified wavenumber regions, *versus* concentration was generated by the Calcmet software and used for subsequent automated Calcmet software determinations of PGME and ethanol concentrations.

Additional samples of PGME in nitrogen were measured at calculated concentrations of 1, 10, 20, 30, 40, and 50 ppmv of PGME in nitrogen as shown in Fig. 4, which produced an R^2 of 1.000. Samples of 1, 9.6, 19, 29 and 39 ppmv of PGME with the presence of a constant

concentration of 30 ppmv ethanol were also measured and produced an R^2 of 0.9991 as shown in Fig. 4. The relative standard deviation (RSD) of the measurement increased with decreasing concentration, which is expected as the signal-to-noise ratio decreases, and other uncertainties increase relative to the delivered PGME concentration. There was a good linear correlation between instrument response and calculated concentration in the range shown.

Several low concentrations of PGME vapor for determination of instrumental LOD and LOQ were generated at concentrations calculated to be 0.100, 0.200, 0.350, 0.675, 1.00 and 1.35 ppmv, with the resulting measured concentrations shown in Fig. 5. The signal from the 0.100 and 0.200 ppmv samples could not be differentiated from blank signal. The linear regression of the samples produced a 1.0226 ppmv/ppmv slope with a standard error of the estimate (SEE) of 0.08412 ppmv. Thus, a calculated instrumental LOD was 0.247 ppmv and the calculated LOQ was 0.823 ppmv of PGME vapor. The LOD is defined as three times the SEE divided by the slope, and the LOQ is ten times the SEE divided by the slope.[19–21]

Concentrations of 0.1, 0.2, 0.35, 0.675, 0.750, 1.00, and 1.35 ppmv PGME in a constant 10 ppmv of ethanol were generated for determining the LOD/LOQ for PGME with 10 ppmv ethanol. The 0.10, 0.20 and 0.35 ppmv concentrations were indistinguishable from the blank. The slope of the linear regression was 0.9046 ppmv/ppmv with an intercept of −0.1170 and the SEE was 0.07768 as shown in Fig. 6. The calculated instrumental LOD was 0.248 ppmv and the calculated LOQ was 0.826 ppmv in 10 ppmv ethanol. The slope being less than one and the negative intercept indicate that the ethanol was an interferent.

We evaluated the uncertainty of instrumental measured concentrations *versus* concentrations calculated from known delivery rates of PGME to create PGME atmospheres. Precision, bias and accuracy of measured concentrations relative to theoretical values delivered by the calibration apparatus were calculated as adapted from Hirst *et al.*[2] Measurements from generated samples at 1, 5, 10, 20 ppmv PGME in nitrogen were used in the results shown in Table 1. The same metrics were evaluated for a mixture of 1, 10, 20, 30, and 40 ppmv PGME with 30 ppmv ethanol in nitrogen.

3.3 Gravimetric analysis of the evaporation of 2.5 M PGME liquid aliquots

Gravimetric analysis of the evaporation of a single drop of 2.5 M PGME aqueous and 2.5 M PGME 30% ethanol aqueous solutions, were plotted as normalized mass *versus* time as shown in Fig. 7. The initial mass of the drops was normalized to account for the influence of the fan on the measurements. Polynomial best fitted curves were drawn to aid in visualization of the trend. The 10 μL drop of the 2.5 M PGME aqueous solution took 20.5 minutes to fully evaporate. The elapsed times for complete evaporation of all volumes of the 30% ethanol solutions were less than for the corresponding volume of the aqueous solutions. The 2 μL drop of the 2.5 M PGME 30% ethanol solution took 5.5 minutes to fully evaporate, which was the minimum time for any of the volumes.

3.4 Analysis of evaporation of 2.5 M PGME in aqueous solutions in the NIOSH chamber

The PGME vapor concentration in the NIOSH chamber was measured by FTIR during the evaporation of 2.5 M PGME solutions from aliquots in the range of 1 μL to 10 μL as shown in Fig. 8. The concentration profile shape was a sharp rise to a peak followed by a decline

in concentration. The decrease after the peak concentration was likely from a combination of losses due to adsorption to the chamber surface, tubing, and instrument. The smallest drop (1 μL) took more than 5 minutes to reach a peak concentration in the NIOSH chamber. The 10 μL drop took 20 minutes or more to reach a peak concentration. The peak concentration was assumed to represent the time of complete evaporation of the drop since the time points of peak concentration correlated well with complete evaporation from the gravimetric analysis in Fig. 7. The chamber was flushed with lab air until the PGME baseline concentration was restored before release of each aliquot.

Example evaporation profiles ($n = 4$) for 10 μL aliquots are shown in Fig. 9. The relationship between PGME vapor concentration and aliquot volumes was evaluated at various time points to determine acceptable analysis points. Evaluations included peak concentration (red arrows), an average concentration of 3 minutes (30 measurements) after the peak of each run (red bracket corresponds to the 30 measurement region of the yellow trace), various time points after the start of evaporation (blue arrows) and the initial slope of the evaporation profile (blue bracket) as shown in Fig. 9.

Peak PGME concentration after release of the drop ($n = 4–5$, 1–10 μL) had a linear relationship as shown in Fig. 10. The linear regression equation had a slope of 0.4031 ppmv μL^{-1} and an intercept of 0.2495 μL with a SEE of 0.1425, resulting in an aliquot volume LOD of 1.06 μL and a LOQ of 3.54 μL.

The average PGME concentration ($[PGME]_{avg}$) was the mean of measurements during the 3 minutes (30 measurements) directly after the peak PGME concentration (Fig. 9, red bracket). A $[PGME]_{avg}$ concentration from 30 measurements after the peak concentration ($n = 4–5$, 1–10 μL) also had a linear regression for aliquots in the range 1–10 μL with an R^2 of 0.987. The percent recovery ($[PGME]_{experimental}/[PGME]_{theoretical} \times 100\%$) for the peak PGME concentration ($[PGME]_{max}$) and average PGME concentration ($[PGME]_{avg}$) are given in Table 2. All the percent recoveries for the measured PGME concentration are within 15% of the theoretical PGME concentration. According to Kennedy *et al.*, recovery should be greater than 75%.[19]

The slope of the evaporation profile for the first 4.5 minutes (45 measurements) (blue bracket, Fig. 9), where the first measurement used was the last zero concentration before the concentration steadily increased, was the least reproducible method with an R^2 of the linear regression slightly above 0.8. PGME vapor concentrations at 3.0, 4.5, 6.0, 7.5, 8.0, and 10.0 minutes showed good linearity during evaporation of drops in the range 2–10 μL, with R^2 above 0.9 from the linear regressions. The 1 μL samples had more variable results, with a theoretical PGME vapor concentration of 0.47 ppmv. All of the measurements at these earlier time points were below the instrumental LOQ of 0.82 ppmv.

3.5 Analysis of evaporation of 2.5 M PGME with 30% v/v ethanol solutions in the NIOSH chamber

Analyses were done of evaporation of 2.5 M PGME with 30% v/v ethanol solutions in the NIOSH chamber in the range 2 μL to 10 μL. Evaporation profiles observed for the 2.5

M PGME solutions with ethanol were similar to the previous evaporation profiles without ethanol.

Peak PGME concentration measurements (n = 4, 2–10 μL) of 2.5 M PGME in 30% ethanol had a linear relationship in the range 2–10 μL with a R^2 of 0.9777 as shown in Fig. 11. The linear regression equation had a slope of 0.3888 ppmv/μL and an intercept of 0.3535 μL with a SEE of 0.1752, resulting in an aliquot volume LOD of 1.35 μL and a LOQ of 4.51 μL. It is important to point out that the 1.35 μL LOD is below the lowest aliquot volume of 2 μL, which does not meet criteria for robust determination of an LOD.

The average $PGME_{avg}$ concentration (30 measurements) after peak concentration (n = 4, 2–10 μL) had a very linear relationship in the range 2–10 μL. The percent recovery ($[PGME]_{experimental}/[PGME]_{theoretical} \times 100\%$) for the $PGME_{max}$ and $PGME_{avg}$ are given in Table 3. All the percent recoveries for the measured PGME were within 13% of the theoretical PGME concentration, except the $PGME_{avg}$ recovery for the 4 μL drop and the $PGME_{max}$ recovery of the 2 μL drop.

The initial evaporation profile slopes (first 4.5 minutes) were more variable for the solutions containing ethanol at all volumes as compared to the purely aqueous solutions. The slope of the evaporation profile for the first 4.5 minutes after release had a linear fit with an R^2 of 0.5. PGME concentration at measurement times 3.0, 4.5, 6.0, 7.5, 8.0, and 10.0 minutes showed good linearity in the range 2–10 μL. However, the fit got progressively worse with the earlier time points. The liquid LOD/LOQ was larger for PGME in the presence of ethanol and the PGME concentrations at the earlier time points were below instrumental LOQ, giving rise to the higher variability.

3.6 Evaluating the number and shape of drops *versus* PGME measurement in the NIOSH chamber

The number of drops or the shape of drops affected the ability to quantitatively correlate measured vapor concentrations to the volume of solution released. PGME vapor concentration at various time points were evaluated to determine acceptable analysis time points. Different numbers or shapes of drops of 2.5 M PGME solution were analyzed *via* FTIR from evaporation in the NIOSH chamber. Drops were dispensed from a gastight syringe either as a single bolus or divided into multiple drops with a total of 4 μL of solution. Aliquots were administered in 1 drop, 2 drops, 3 drops, or a single drop which was smeared, with four replicates for each. A single replicate is shown in Fig. 12, demonstrating the concentration profiles observed for various drops.

The rate of PGME concentration increase was greatest for the smeared drop, followed by 3 drops, then 2 drops, and then 1 drop. The peak PGME concentration and average PGME concentration were unaffected by the number of drops or shape of the drop. The RSD of the measurements was 6.67% for the maximum PGME concentration and 7.38% for the average PGME concentration. The change in concentration *versus* time (slope during the first 4.5 minutes) was very dependent on the number and shape of the drops.

For evaporation of 2.5 M PGME 30% ethanol (aq) solution, the maximum PGME concentration and average PGME concentration were unaffected by the number of drops or shape of the drop. The RSD of the concentration measurements were 9.58% for the peak PGME concentration and 7.21% for the average PGME concentration.

3.7 Evaluating the release of PGME headspace in the NIOSH chamber

In addition to liquid leaks, headspace containing PGME may also be released during use of CSTDs. Transfer of solutions between vials may result in displacement of a headspace volume equivalent to the amount of liquid transferred when using air-filtering CSTDs. The theoretical amount of PGME in a volume of headspace can be calculated. The published experimental value of Henry's constant is, $H_{\mathrm{v}}^{\mathrm{pc}} = 9.20 \times 10^{-7}$ atm m^3 mol^{-1}.[22] The equations used to calculate the vial headspace concentration of PGME were adapted from R. Sander.[23]

Solving for the concentration of PGME in headspace above a 2.5 M aqueous PGME was done using Henry's constant $\left(H_{\mathrm{v}}^{\mathrm{pc}}\right)$, the ideal gas constant (R), and temperature (T) as shown in the following equation.

$$\frac{H_{\mathrm{v}}^{\mathrm{pc}} \times \text{liquid concentration}}{RT} = \text{gas concentration}$$

$$\frac{\left(9.20 \times 10^{-7}\ \text{atm m}^3\ \text{mol}^{-1}\right)(2.5\ \text{M PGME})}{\left(8.21 \times 10^{-5}\ \text{m}^3\ \text{atm mol}^{-1}\ \text{K}^{-1}\right)(295.35\ \text{K})}$$

$$= 9.5 \times 10^{-5}\ \text{M PGME(g)}$$

For a volume of 45 mL of headspace above a 2.5 M PGME solution, released into the NIOSH chamber at conditions of 22.2 °C, 98.4 kPa, and a chamber volume of 131 L would theoretically produce a vapor concentration of 0.8 ppmv. If the headspace were displaced by liquid, then a dilution of the headspace would not occur and the concentration in the chamber would be the theoretical 0.8 ppmv for a 45 mL displacement. This 0.8 ppmv concentration would be just below the instrumental LOQ of 0.823 ppmv. Releases of less than 45 mL of headspace, or if a filter had some efficacy that reduced the PGME in the effluent, would result in PGME concentration in the chamber that may be well below the LOQ.

If headspace from a vial was introduced into the chamber by displacement with clean air, the headspace would be diluted in the process of displacement. A first order kinetics of dilution model was used to calculate released headspace concentrations, where h is the fraction of the molecules in the headspace that are displaced after injecting a displacement volume, v_{d} from an initial headspace volume, v_{i} and e is Euler's number.

$$h = 1 - e^{-\left(\frac{v_{\mathrm{d}}}{v_{\mathrm{i}}}\right)}$$

It would be expected that approximately 63% of the PGME molecules in the headspace at equilibrium will become part of the effluent when injecting a volume of air equal to the headspace volume. This model neglects volatility of the PGME from solution to reestablish

headspace equilibrium during the displacement. We assume the establishment of PGME headspace equilibrium as $\Delta C/\Delta t$ (change in concentration with change in time) is slow compared to the change in concentration by displacement using air from the syringe.

We evaluated the concentration of PGME in the NIOSH chamber after using a syringe and needle to inject air into the headspace of a 100 mL drug vial, sealed with a crimped cap septum, and containing 50 mL of 2.5 M PGME aqueous solution (an additional needle was inserted into the septum to provide a route for displaced headspace effluent). Injection of 50 mL of lab air into a vial was repeated five times, with increases in PGME concentration observed after each injection as shown in Fig. 13. It was expected that about 0.57 ppmv of PGME would be in the chamber after displacing 50 mL of headspace from the vial with lab air. The measured PGME concentration after each injection was averaged (red line, Fig. 13), with the change in concentration listed in Table 4. Time between injections was 10.33 min (between 1 & 2), 8.33 min (between 2 & 3), 7 min (between 3 & 4), and 7 min (between 4 & 5). During injection 4, the effluent needle became clogged, by coring of the septum, and the injected air was trapped inside the vial. The trapped air and the subsequent injection was released during the 5^{th} injection.

From a vial containing 50 mL of 2.5 M PGME 30% ethanol aqueous solution, the displacement process was repeated five times as shown in Fig. 14. Increases in measured PGME concentration after each injection of air are listed in Table 5. The more consistent increase in measured PGME from the headspace above the ethanol solution *versus* the aqueous solution could be due a shorter equilibrium time to reestablish the PGME headspace in the presence of ethanol. Time between injections was 7 min (between 1 & 2), 5.5 min (between 2 & 3), 6.25 min (between 3 & 4), and 6.5 min (between 4 & 5).

3.8 Effect of humidity on PGME measurement in the NIOSH chamber

Nitrogen was used to purge humidity from the NIOSH chamber. We compared concentration measurements from the evaporation of 4 μL aliquots of 2.5 M PGME solution released in the chamber as single drops. FTIR measurements were of the PGME concentration in the NIOSH chamber containing lab air (1% v/v water), partially nitrogen-purged (0.3% v/v water) and nitrogen-purged (0.05% v/v water). The water content during purging of the chamber with N_2 was measured using the FTIR analyzer. The nitrogen-purged chamber (0.05% water) significantly reduced the time to reach peak PGME concentration, as shown in Fig. 15. However, the observed average ($n = 3$) peak concentration of 0.857 ppmv was significantly lower than the theoretical value of 1.9 ppmv from a 4 μL aliquot.

Concentration measurements during the evaporation of a 4 μL drop of the 2.5 M aqueous PGME solution in the chamber that was partially purged with nitrogen is also shown in Fig. 15. The ($n = 1$) peak PGME concentration was 0.99 ppmv. Peak concentration of PGME changed with reduced humidity. We theorized that this was due to increased adsorption of PGME to the chamber walls at lower humidities. Low humidities negatively impact the observed PGME vapor concentration and the liquid volume LOQ.

4. Conclusions

Liquid leaks were accurately measured using the peak instrument response after complete evaporation of the droplets. The measurement at peak concentration was the most robust time point for correlating measured vapor concentration to volumes of liquid aliquots. This method demonstrates relatively good correlation between peak concentration in the NIOSH chamber for leak volumes from 2 μL up to 10 μL of 2.5 M PGME solution with and without the presence of 30% ethanol. For a 2.5 M PGME aqueous solution release in the NIOSH chamber, the calculated LOQ was 3.5 μL and the LOD was 1.0 μL. The presence of ethanol negatively affects the LOD and LOQ. For 2.5 M PGME solutions containing 30% v/v ethanol, the LOQ was 4.5 μL and the LOD was 1.4 μL.

Complete evaporation of liquid droplets was slow, compromising the ability to correlate a leak with a particular manipulation of a CSTD. To achieve the best correlation between PGME solution volume and instrument response, it was imperative to wait for peak PGME concentration. The peak concentration can take up to 9 min for a 2 μL drop and up to 20 min for a 10 μL drop of solution. In contrast to testing procedures using a more volatile surrogate, which could distinguish leaks as an almost immediately measurable response, real-time correlations of measured PGME and manipulations of the CSTD may not be discernable given the evaporation time for PGME.

Quantification of liquid leak volume before complete evaporation of droplets was not robust. Analysis was done of the PGME concentration at several time points before complete evaporation of single drops of various volumes. At time points between 6 and 8 minutes, the linear regression of the concentration *versus* volume for both aqueous and 30% ethanol aqueous solutions produced an $R^2 = 0.81$ and $R^2 = 0.86$ for 6 and 8 min, respectively, in aqueous, and $R^2 = 0.76$ and $R^2 = 0.87$ for 6 and 8 min, respectively, for 30% ethanol solutions. These time points occurred before complete evaporation of the sample and were not well correlated with the final magnitude of a leak.

The method has a very narrow quantitative range. Complete evaporation of droplets was necessary for robust quantitation of liquid releases. Therefore, the slow evaporation of larger droplets places a practical upper limit on the drop size that can be measured in a reasonable period of time. A 10 μL drop, the largest evaluated, requires up to 20 minutes for complete evaporation. The lower end of the quantifiable range as determined by the method LOQ is 3.5 μL. Even if measurement of somewhat larger drops is considered viable, the method has a very narrow quantitative range for liquid droplets.

Liquid leaks consisting of different numbers of droplets or different shapes evaporate at different rates. The number of drops and shape had significant effects upon the relationship between measured PGME vapor concentrations and aliquot volume. The measured PGME concentration varied depending on drop size and shape unless the peak concentration was used. The measurements before peak concentration, extrapolated to the total concentration, would likely not be robust enough for real systems.

Adsorption of PGME within the chamber reduced peak concentration and adds variability to the measurement. Adsorption of PGME to the chamber seems to be a significant

factor, demonstrated by time *versus* concentration plots where the concentration decreases after reaching a peak concentration. Adsorption in the chamber means that the maximum concentration measured may be an underestimation of the actual leaked amount of PGME. Multiple leaks that occur at separate time points during testing may confound a measurement of peak concentration being correlated with a volume due to issues of adsorption of PGME to the chamber walls.

Humidity of the air within the chamber affected the rate of evaporation and the peak concentration. When lab air containing water vapor was replaced by dry nitrogen, the rate of droplet evaporation (as measured by the time to peak concentration) increased. The peak concentration was significantly reduced by purging the chamber with nitrogen. This was interpreted as a peak concentration dependent on humidity. Presumably this was due to increased adsorption of PGME to the chamber walls at lower humidities.

Headspace leaks likely to be encountered in the NIOSH testing protocol will be below the LOQ of the method. Headspace volumes of 45 mL from above a 2.5 M PGME solution would theoretically produce a 0.8 ppmv concentration in the chamber, when displaced by liquid, which is close to the instrumental LOQ of 0.82 ppmv. Volumes smaller than 45 mL and partial removal of PGME by filters would result in PGME concentrations below the instrumental LOQ in the NIOSH chamber.

This method cannot distinguish between liquid leaks and headspace leaks. Quantifying the total PGME released into the chamber does not enable a liquid leak to be distinguished from a headspace leak. Because PGME partitions into the headspace to a greater extent than most if not all hazardous drugs, measurement of PGME originating from a headspace leak will overestimate the amount of hazardous drug that would have been present in the same volume of headspace.

The most influential and easily achievable improvements to the capabilities of this test involve improving the limits of detection for the system. Without changing the FTIR detector or challenge agent (PGME), the size of the testing chamber would have to be significantly decreased, which would allow for improved LOD/LOQ to address the current limitation in quantifying the PGME from headspace leaks. However, this could compromise the ability to perform real world manipulations of CSTD systems, such as transferring a drug from one vial to another. A secondary breakthrough test that uses a much smaller chamber to challenge CSTDs with PGME vapor would enable evaluation of the efficacy of filter type CSTDs to retain PGME vapor.

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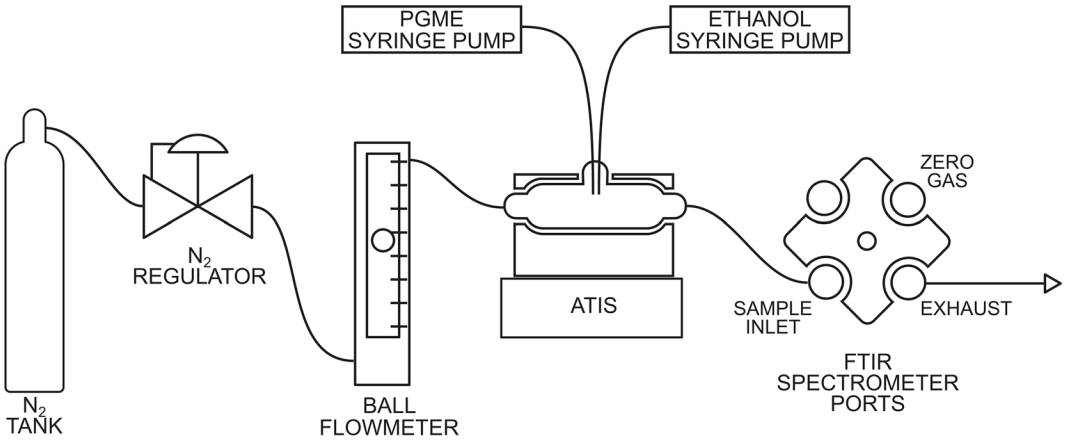

Fig. 1.

Calibration apparatus connections where nitrogen gas metered from a regulator flowed into a ball flow meter, then into the ATIS where it was mixed with PGME and/or ethanol from syringe pumps. The effluent from the ATIS was routed to the FTIR sample inlet port.

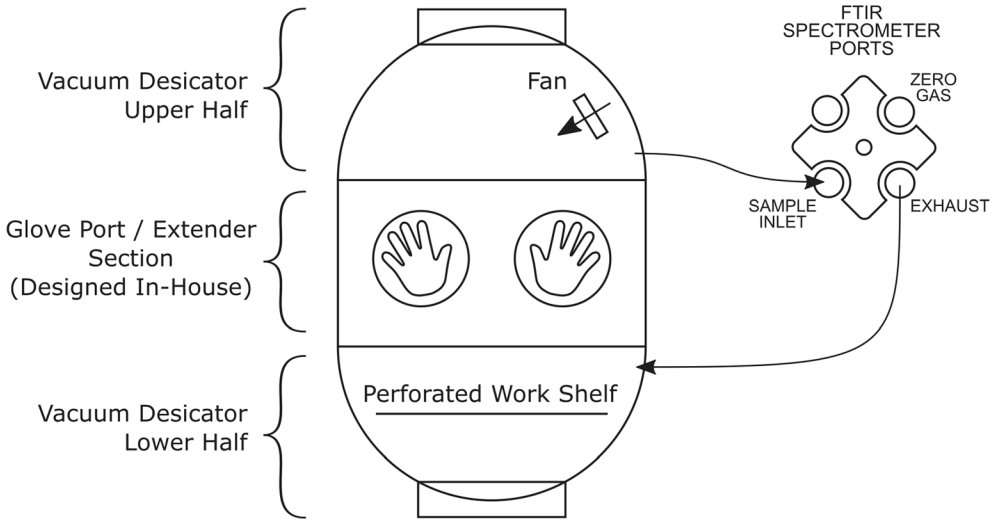

Fig. 2.

NIOSH chamber depicting the configuration including location of the fan, glove ports, work shelf, and connections to the FTIR spectrometer.

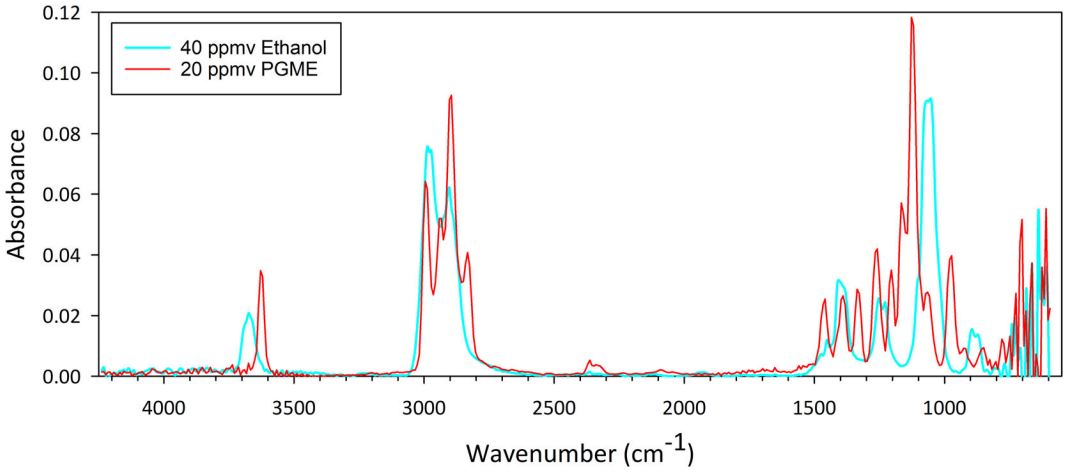

Fig. 3.

FTIR spectrum of 20 ppmv PGME vapor in nitrogen, generated using the calibration apparatus. Overlayed spectrum of 40 ppmv ethanol in nitrogen included so that the overlapping regions of absorbance are observable.

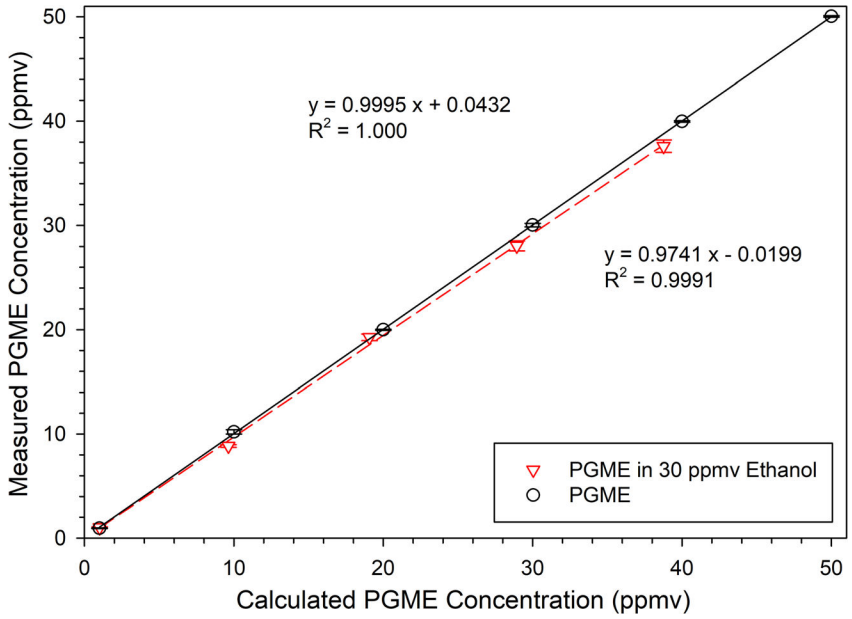

Fig. 4.

Average measured concentration of PGME in nitrogen with and without the presence of 30 ppmv ethanol *versus* calculated PGME concentration generated using the calibration apparatus. The graph demonstrates instrumental linearity.

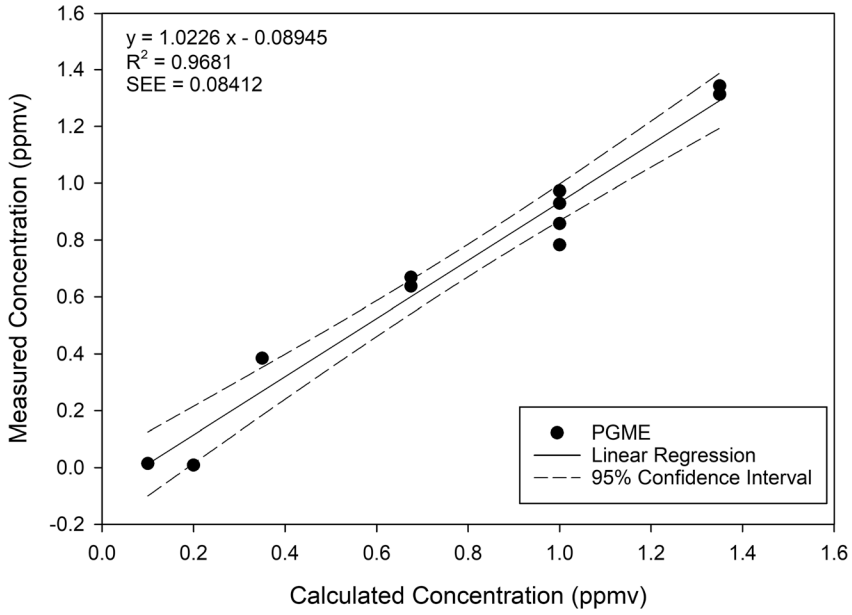

Fig. 5.

Measured concentration *versus* calculated concentration of PGME in nitrogen generated in the calibration apparatus for determination of instrumental detection limits.

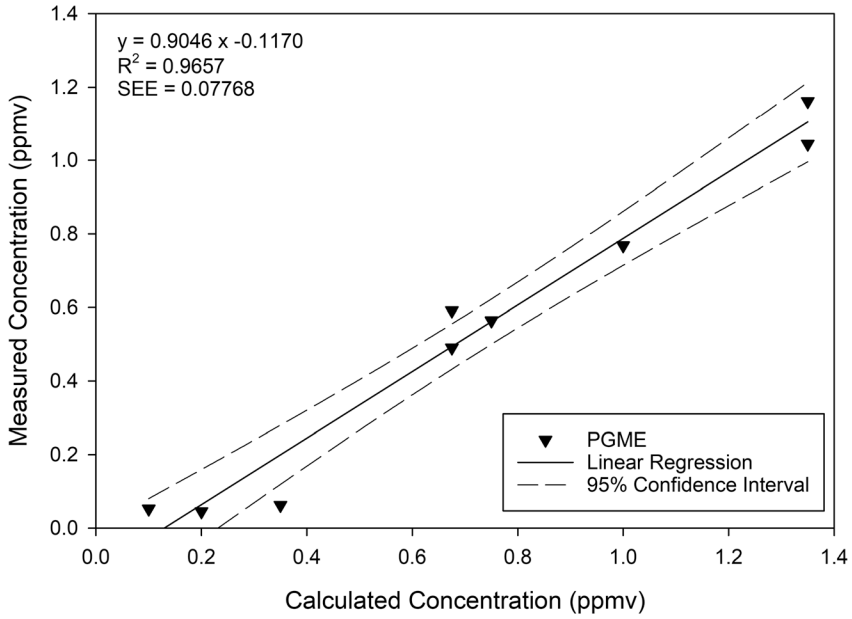

Fig. 6.

Measured concentration of PGME *versus* calculated concentration in a constant 10 ppmv ethanol in nitrogen generated using the calibration apparatus for determination of instrumental detection limits.

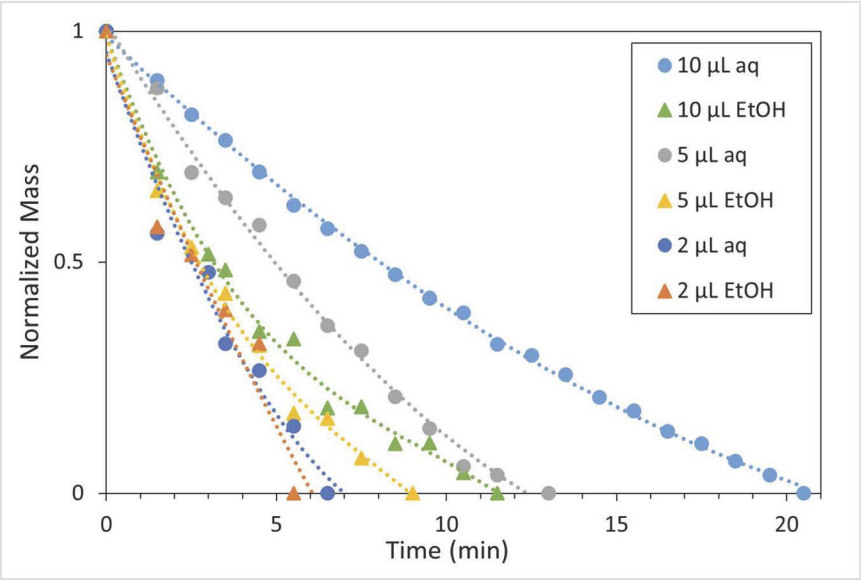

Fig. 7.

Gravimetric record of the evaporation of 2.5 M PGME aqueous (aq) and 2.5 M PGME 30% ethanol aqueous (EtOH) solutions shown as normalized mass *versus* time.

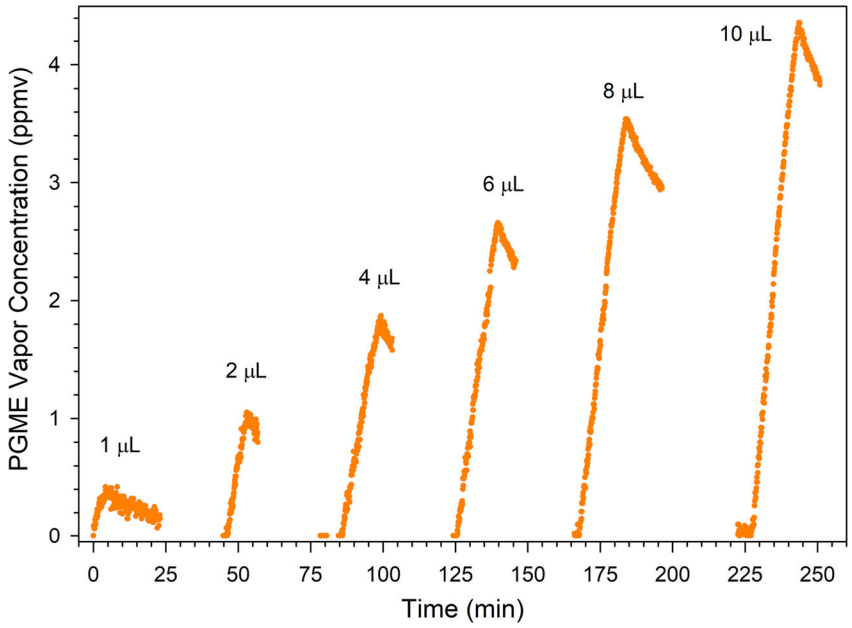

Fig. 8.

FTIR measurements of PGME vapor concentration *versus* time, from releases of aliquots of 2.5 M PGME solution evaporated in the NIOSH chamber. Between aliquots, PGME was flushed from the chamber and concentrations could return to baseline before the next aliquot.

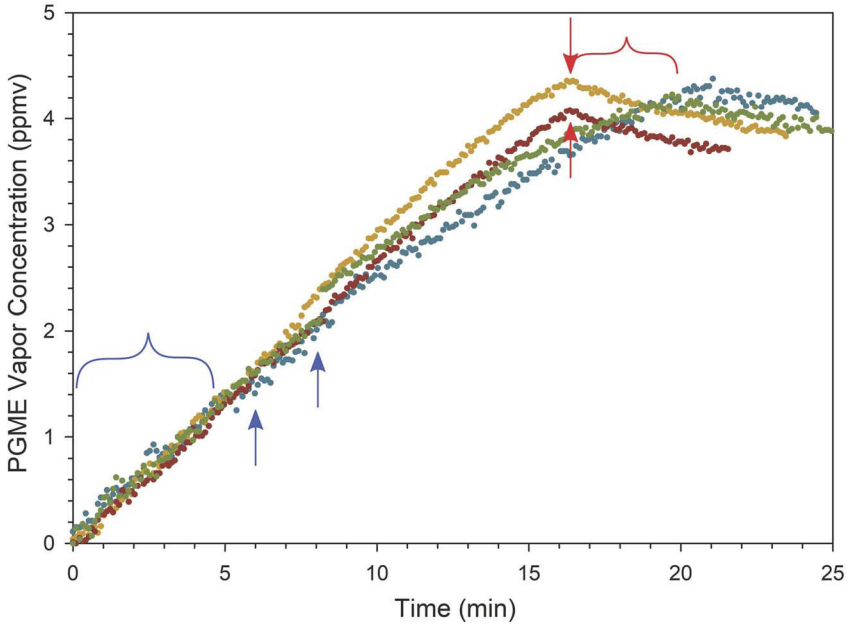

Fig. 9.

Evaporation profile of four replicate measurements of 10 μL drops of 2.5 M PGME, evaporated in the NIOSH chamber at room temperature with a fan running, shown as PGME concentration (ppmv) *versus* time. The maximum PGME concentration (red arrows for the red and yellow lines), average PGME concentration of the 30 points after the peak (red bracket for the yellow line), range of slope of evaporation profile (blue bracket), and specific measurement time points (blue arrows at 6 and 8 minutes) are indicated on the figure.

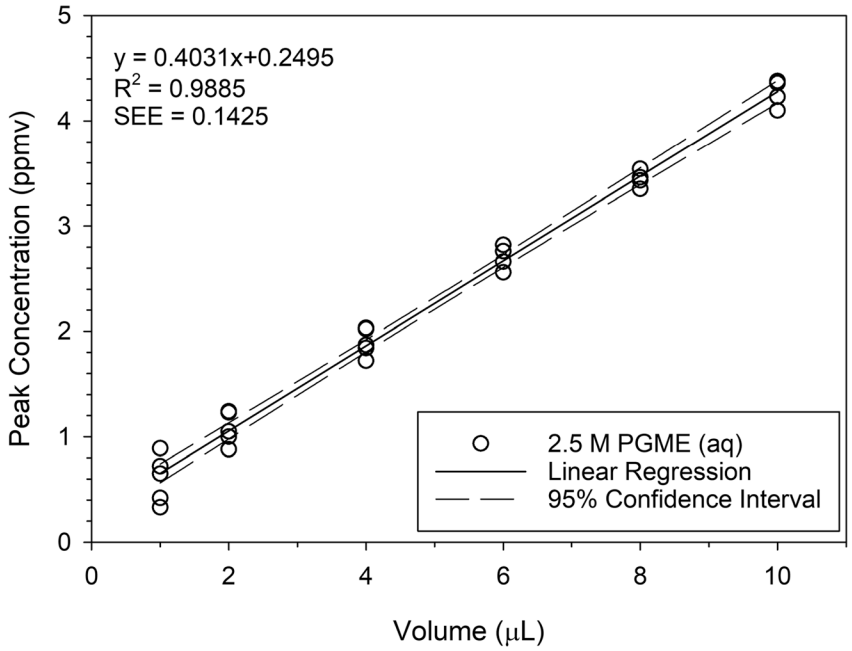

Fig. 10.

FTIR measurements of peak PGME concentration *versus* drop volume (μL) of 2.5 M PGME in water, evaporated in the NIOSH chamber. Each volume was measured with four (for 5–10 μL) or five (for 1–4 μL) replicates.

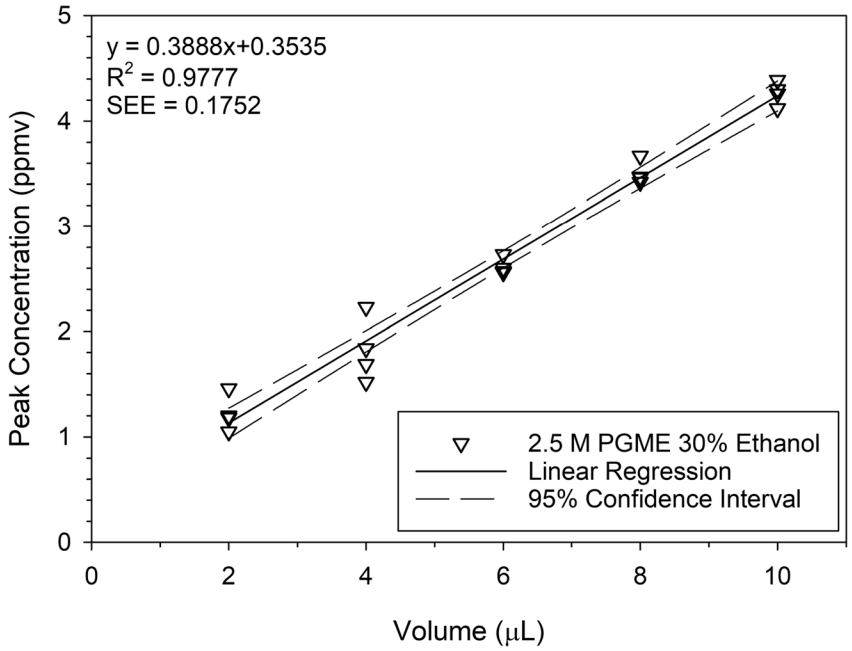

Fig. 11.

FTIR measurements of peak PGME concentration *versus* drop volume of 2.5 M PGME with 30% v/v ethanol.

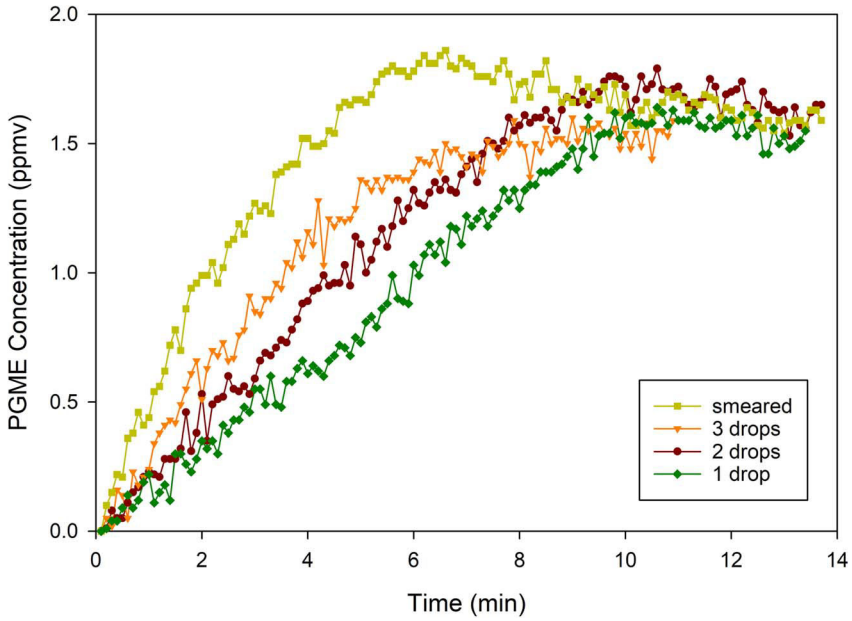

Fig. 12.

PGME concentration *versus* time. The traces generally increase linearly, then reached a peak PGME concentration followed by a decrease in PGME concentration.

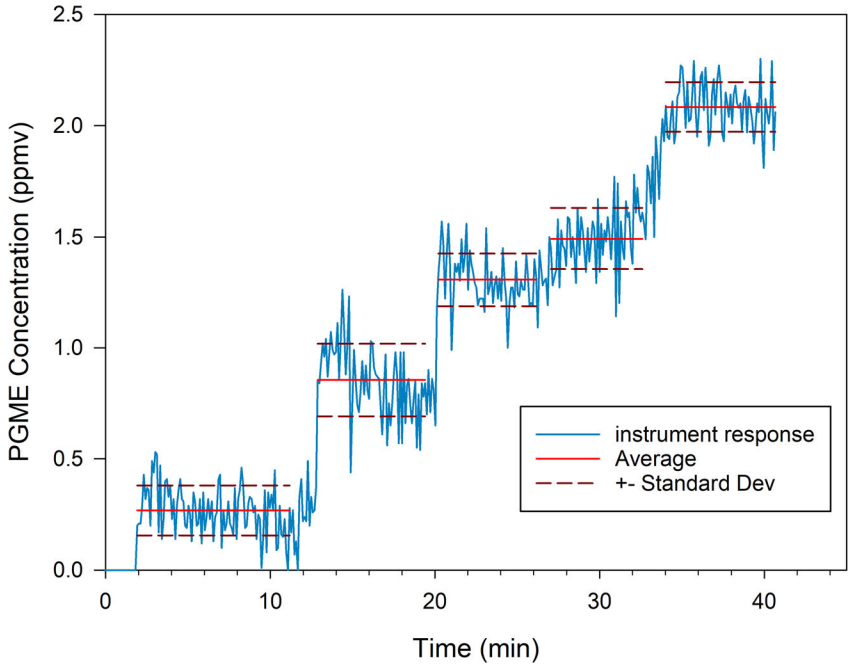

Fig. 13.

Instrument response due to injection of air to displace 50 mL of headspace above a 2.5 M PGME solution. Shown is a single experiment where the average and standard deviation of PGME concentrations after each of five displacements of headspace during continuous measurement in a closed chamber were calculated.

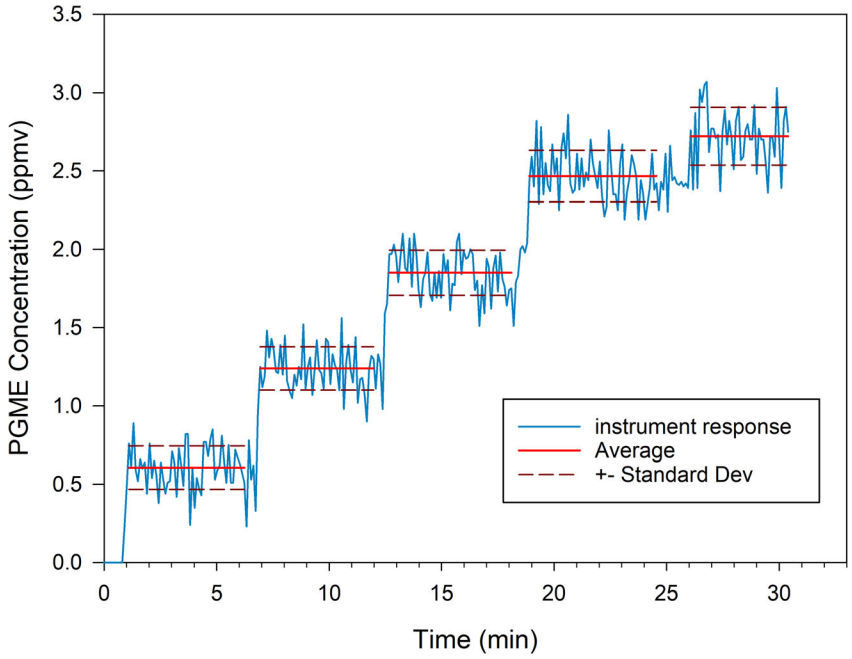

Fig. 14.

Changes in concentration of PGME in the NIOSH chamber due to displacing 50 mL of headspace above a 2.5 M PGME 30% ethanol aqueous solution. Shown is a single experiment where the average and standard deviation of PGME concentrations after each of five displacements of headspace during continuous measurement in a closed chamber were calculated.

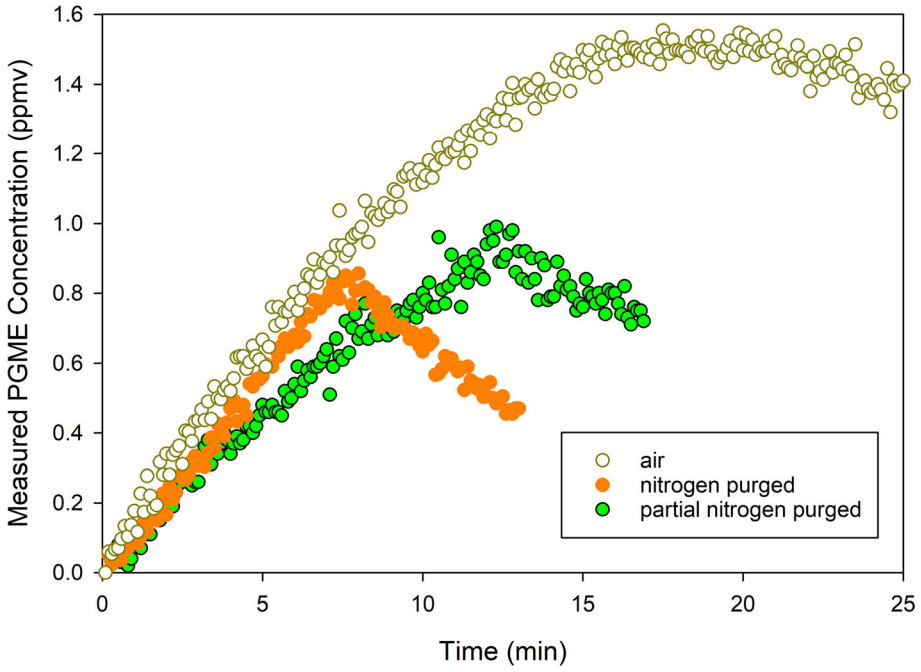

Fig. 15.

Average PGME concentration *versus* time from the evaporation of a 4 μL drop of 2.5 M aqueous PGME in the nitrogen-purged, partial-nitrogen-purged, and lab air filled NIOSH chamber. Air and nitrogen purged data are an average of 3 independent experiments. The partial nitrogen purged data is from a single experiment.

Table 1

Precision, bias, and accuracy for measurements of vapors of PGME and PGME with ethanol in nitrogen

	PGME in N_2	**PGME & ethanol in N_2**
Precision	3.55%	3.29%
Bias	−2.49%	−2.41%
Accuracy	8.32%	7.82%

Table 2

Average PGME concentration and percent recoveries for drops of 2.5 M PGME in water

Drop volume (μL)	$[PGME]_{theor}$ (ppmv)	Avg $[PGME]_{max}$ (ppmv) ± SD	$[PGME]_{max}$ recovery (%) ± SD	Avg $[PGME]_{avg}$ (ppmv) ± SD	$[PGME]_{avg}$ recovery (%) ± SD
10	4.70	4.27 ± 0.13	90.9 ± 2.5	4.09 ± 0.09	87.1 ± 2.0
8	3.75	3.44 ± 0.08	90.6 ± 3.5	3.30 ± 0.12	87.8 ± 3.4
6	2.81	2.70 ± 0.11	95.1 ± 6.1	2.56 ± 0.16	91.3 ± 5.6
4	1.88	1.90 ± 0.13	100.6 ± 6.6	1.77 ± 0.12	94.4 ± 6.2
2	0.94	1.08 ± 0.15	114.6 ± 16.3	0.99 ± 0.16	105.2 ± 16.9
1	0.47	0.602 ± 0.23	128.1 ± 48.3	0.508 ± 0.23	108.1 ± 48.0

Table 3

Average PGME concentration and percent recoveries for drops of 2.5 M PGME 30% ethanol solutions

Drop volume (μL)	$[PGME]_{theor}$ (ppmv)	Avg $[PGME]_{max}$ (ppmv) ± SD	$[PGME]_{max}$ recovery (%) ± SD	Avg $[PGME]_{avg}$ (ppmv) ± SD	$[PGME]_{avg}$ recovery (%) ± SD
10	4.77	4.27 ± 0.11	89.5 ± 2.4	4.17 ± 0.13	87.4 ± 2.8
8	3.80	3.50 ± 0.11	92.3 ± 3.4	3.35 ± 0.16	89.5 ± 2.9
6	2.85	2.62 ± 0.08	91.8 ± 3.2	2.51 ± 0.05	88.1 ± 2.0
4	1.90	1.82 ± 0.30	95.9 ± 15.9	1.54 ± 0.16	81.4 ± 8.7
2	0.95	1.22 ± 0.17	128.7 ± 18.1	0.95 ± 0.15	106.0 ± 11.4

Table 4

Average PGME concentration, standard deviation and increase for each injection of air to displace headspace from a vial containing 2.5 M PGME aq

Injection #	PGME avg ± Std. Dev. (ppmv)	PGME increase (ppmv)
1	(0.27 ± 0.1)[a]	0.27[a]
2	0.86 ± 0.16	0.59
3	1.31 ± 0.12	0.45
4	1.49 ± 0.14[b]	0.19[b]
5	2.08 ± 0.11	0.77 (0.39)[c]

[a]Below LOQ.

[b]Clogged effluent needle, the release of [PGME] was less than expected.

[c]Difference between injection 5 and 3 (difference divided by 2 to approximate increase after single injection).

Table 5

Average PGME concentration, standard deviation and increase for each injection of 50 mL of air into a vial containing 2.5 M PGME with 30% ethanol

Injection #	[PGME] avg ± Std. Dev. (ppmv)	[PGME] increase (ppmv)
1	(0.61 ± 0.14)[a]	0.61[a]
2	1.24 ± 0.14	0.63
3	1.85 ± 0.14	0.61
4	2.47 ± 0.16	0.62
5	2.72 ± 0.18[b]	0.25[b]

[a]Below LOQ.

[b]Clogged needle, the rise in [PGME] was less than expected.