

SIMPLE, RAPID ANALYSIS OF ETHYLENE OXIDE IN A POLYSORBATE 80 EXCIPIENT USING SIFT-MS

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Quantitative ethylene oxide analysis in Polysorbate 80 excipient is greatly simplified using SIFT-MS, with a time to first test result that is eight-fold faster than the current compendial method and a daily sample throughput that is 9- to 14-fold higher.

Abstract

Ethylene oxide is a toxic volatile organic compound (VOC) that occurs in various polyethylene glycol (PEG) products – such as Polysorbate 80 – as a manufacturing residue. Due to the toxicity of ethylene oxide (a well-known sterilant), PEG-based excipients must be tested to ensure it is at safe levels. Although conventional compendial methods utilizing gas chromatography (GC) are effective, they involve protracted sample preparation and slow chromatographic separation steps. This means that the time to first test result is slow, and the sample throughput is relatively low.

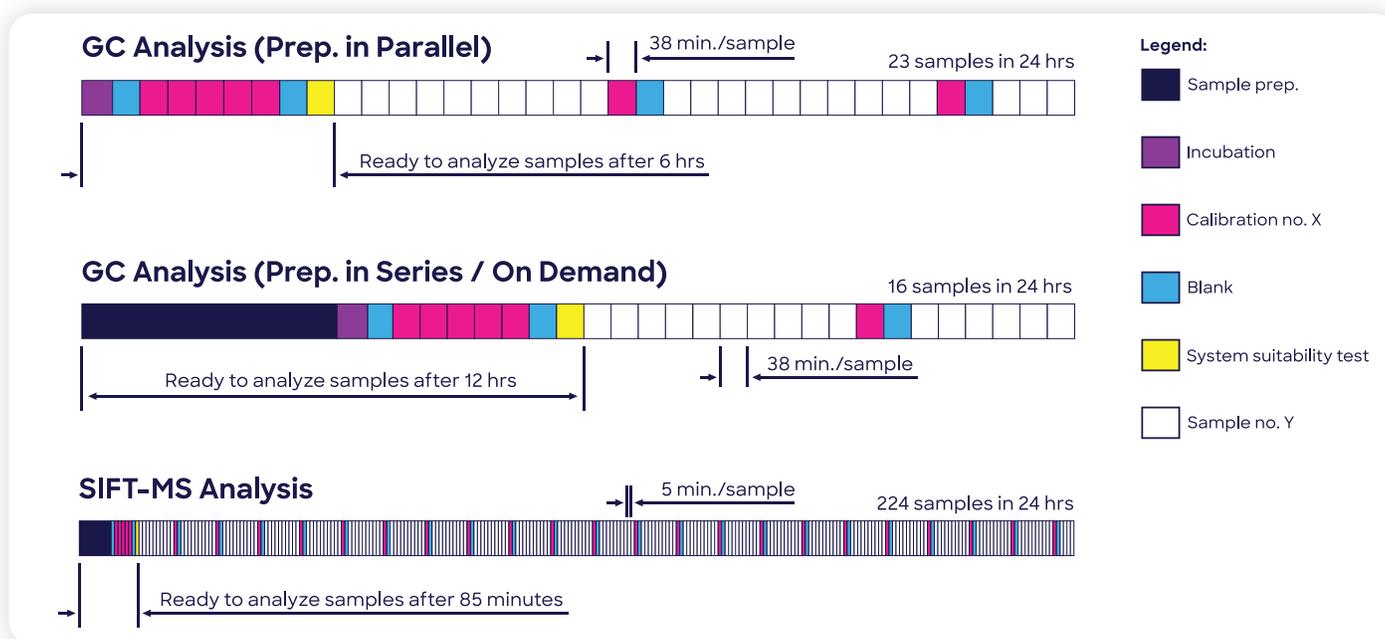
Selected ion flow tube mass spectrometry (SIFT-MS) effectively addresses these limitations through elimination of the long sample preparation step and significantly higher sample throughput. Headspace SIFT-MS analysis is over eight times faster than GC in providing the first test result, and – depending on how sample prep is conducted – has 9- to 14-fold higher daily throughput than GC. These efficiencies are gained while maintaining high analytical sensitivity, using 100-fold less Polysorbate 80 sample per analysis.

INTRODUCTION

Ethylene oxide (EtO) is a widely used feedstock in the chemical industry (Wikipedia 1), including in the manufacture of polyethylene glycols (PEGs) used as surfactants and emulsifiers perfumes, cosmetics, and pharmaceuticals. It is also used widely as a sterilant in the pharmaceutical and medical implants industries because it bactericidal, sporicidal, and virucidal, while being compatible with a wide range of biomaterials with which radiation and heat sterilization are incompatible (Qiu et al. (2017)). However, the toxicity is not limited to microbes: it is mutagenic to humans (Aronson (2016)). For this reason, detection of trace ethylene oxide impurities is essential.

Polysorbate 80 is a polyethylene oxide product used as an emulsifier in various pharmaceuticals and is available under various trade names (Wikipedia 2), including Tween 80®. The United States Pharmacopeia (USP) monograph Polysorbate 80 (USP (2015)) describes the conventional gas chromatography-flame ionization detection (GC-FID) method for EtO analysis. This approach is slow both from the perspective of sample preparation (USP (2013)) and sample analysis. The rate-limiting step in sample preparation is the 6-hr purification of the PEG matrix for matching to the sample (1 mL of Polysorbate 80 in 1 mL of N,N-dimethylacetamide and 0.2 mL of water). Further, the GC-FID run time is 38 minutes per sample.

Figure 1. Headspace SIFT-MS enables analysis of ethylene oxide in Polysorbate 80 (Tween 80®) to be conducted at significantly higher throughput, while eliminating very slow sample preparation due to enhanced sensitivity.



In this application note, a simplified alternative analytical approach is postulated. By leveraging the higher sensitivity of headspace analysis coupled with selected ion flow tube mass spectrometry (SIFT-MS), a much smaller quantity of Polysorbate 80 needs to be used compared to headspace GC-FID. This removes the need for matrix matching and therefore eliminates the need for the 6-hr PEG purification step. Additionally, headspace-SIFT-MS analysis of ethylene oxide in Polysorbate 80 delivers a nine-fold higher sample throughput than GC-FID and hence a much faster time to first test result when calibrations, blanks, and a system suitability test (SST) are considered (85 min. compared to 6 hours; see Figure 1).

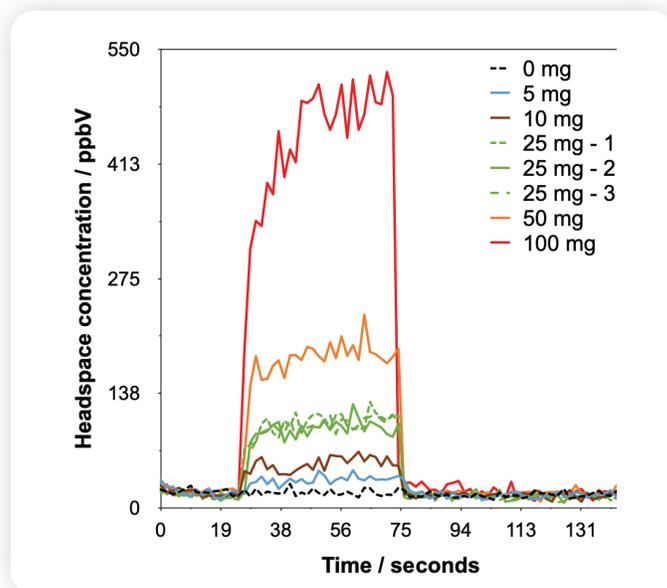
Method

1. The SIFT-MS technique

This work utilized a Syft Technologies Voice200ultra SIFT-MS instrument operating on helium carrier gas. SIFT-MS (Figure 2) uses soft chemical ionization (CI) to generate mass-selected reagent ions (Smith et al. (2020)) that can rapidly react with and quantify VOCs down to part-per-trillion concentrations (by volume, pptV). Up to eight reagent ions (H_3O^+ , NO^+ , O_2^+ , O^- , OH^- , O_2^- , NO_2^- and NO_3^-) obtained from a microwave discharge in air are now applied in commercial SIFT-MS instruments (Hera et al. (2017)). These reagent ions react with VOCs and other trace analytes in well-controlled ion-molecule reactions, but they do not react with the major components of air (N_2 , O_2 and Ar). This enables direct, real-time analysis of air samples to be achieved at trace and ultra-trace levels without pre-concentration. Rapid switching between reagent ions provides high selectivity because the multiple reaction mechanisms give independent measurements of each analyte. The multiple reagent ions frequently remove uncertainty from isobaric overlaps in mixtures containing multiple analytes.

Automated MHE analysis was carried out using a SIFT-MS instrument coupled with a multipurpose autosampler (MPS Robotic Pro, GERSTEL; Mülheim, Germany). The autosampler was controlled using GERSTEL's Maestro software. Samples were incubated at 80 °C for 45 min. in a GERSTEL agitator. Headspace

Figure 3. Example headspace injections with synchronous SIFT-MS analysis of ethylene oxide detected from Tween 80® at various dilution levels in water.



was sampled using a 2.5-mL headspace syringe (heated to 150 °C) and subsequently injected at a flow rate of 50 $\mu\text{L s}^{-1}$ into the SIFT-MS instrument's autosampler inlet (heated to 150 °C) via a self-sealing GERSTEL septumless sampling head. Since the nominal sample flow into the SIFT-MS instrument is 420 $\mu\text{L s}^{-1}$, a make-up gas flow (ultra-high purity nitrogen) is also introduced through the sampling head. This dilution is automatically accounted for in the calibration procedure below. The analysis time for each sample was 145 s (Figure 3) and reported concentrations are the mean of the values obtained during injection (i.e., between ca. 40 and 70 s). Note that no internal standard was utilized (Perkins and Langford (2021a)).

1. SIFT-MS detection of ethylene oxide and discrimination from acetaldehyde

SIFT-MS readily detects ethylene oxide, as shown in Table 1. Reaction rate coefficients, k , are the primary measure of SIFT-MS sensitivity and it is evident that the NO^+ reagent ion is significantly less sensitive to

Figure 2. Schematic diagram of SIFT-MS - a direct, chemical-ionization analytical technique.

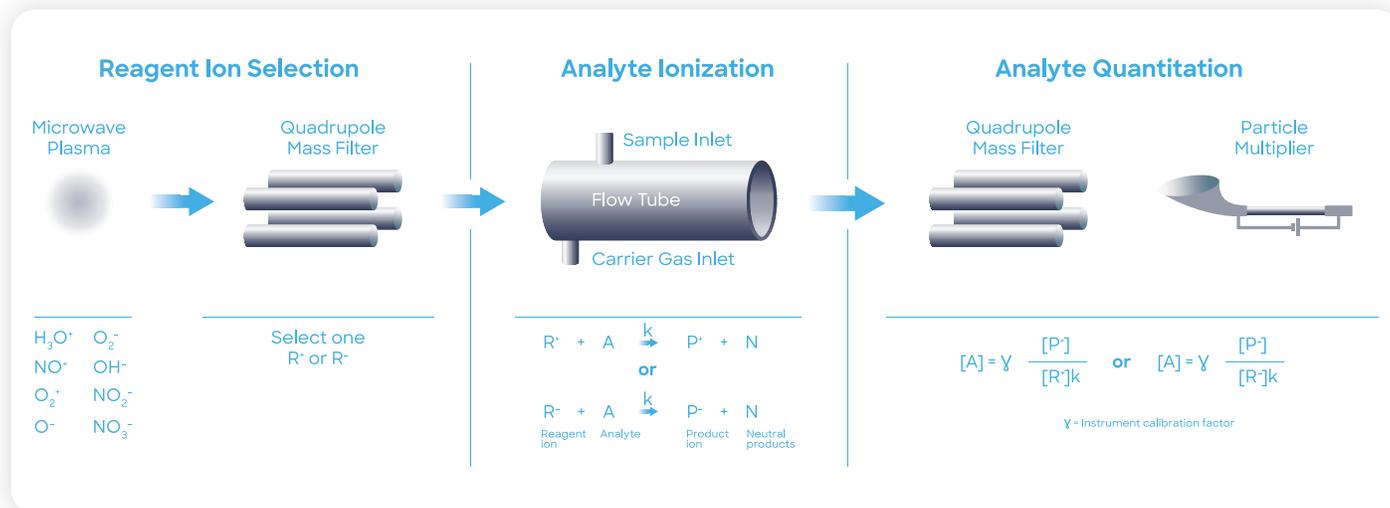
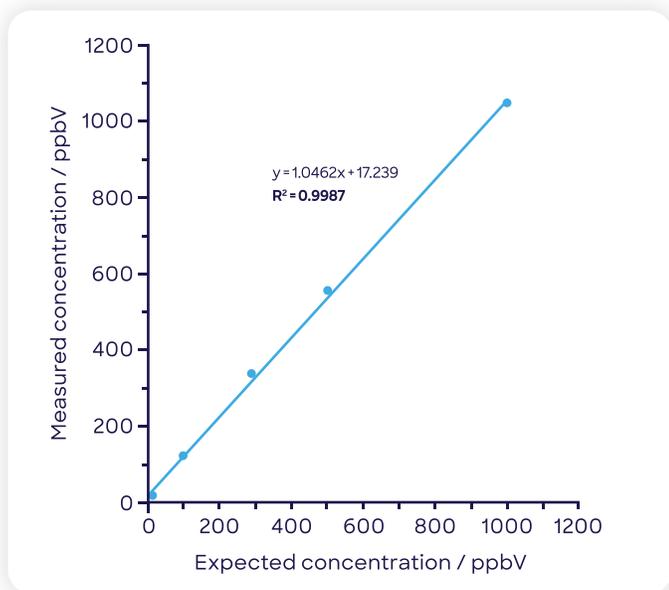


Table 1. SIFT-MS reaction chemistry (rate coefficient (*k*), product ion formulae, branching ratio (BR as %), and mass-to-charge ratio (*m/z*)) for ethylene oxide (EtO) and acetaldehyde, a potential interferent. Ions in gray are not ordinarily used for quantitation because they are frequently occurring product ion *m/z* for other VOCs.

Reagent Ion	Ethylene oxide (EtO)				Acetaldehyde			
	<i>k</i>	Formula	BR	<i>m/z</i>	<i>k</i>	Formula	BR	<i>m/z</i>
H ₃ O ⁺	2.4x10 ⁻⁹	C ₂ H ₄₂ O.H ⁺	100%	75	3.7x10 ⁻⁹	C ₂ H ₄ O.H ⁺	100%	45
NO ⁺	0.10x10 ⁻⁹	C ₂ H ₃ O.NO ⁺	100%	74	0.60x10 ⁻⁹	C ₂ H ₃ O ⁺	100%	43
O ₂ ⁺	2.0x10 ⁻⁹	C ₂ H ₄ O ⁺	100%	44	2.3x10 ⁻⁹	C ₂ H ₄ O ⁺	55%	44
						C ₂ H ₃ O ⁺	45%	43

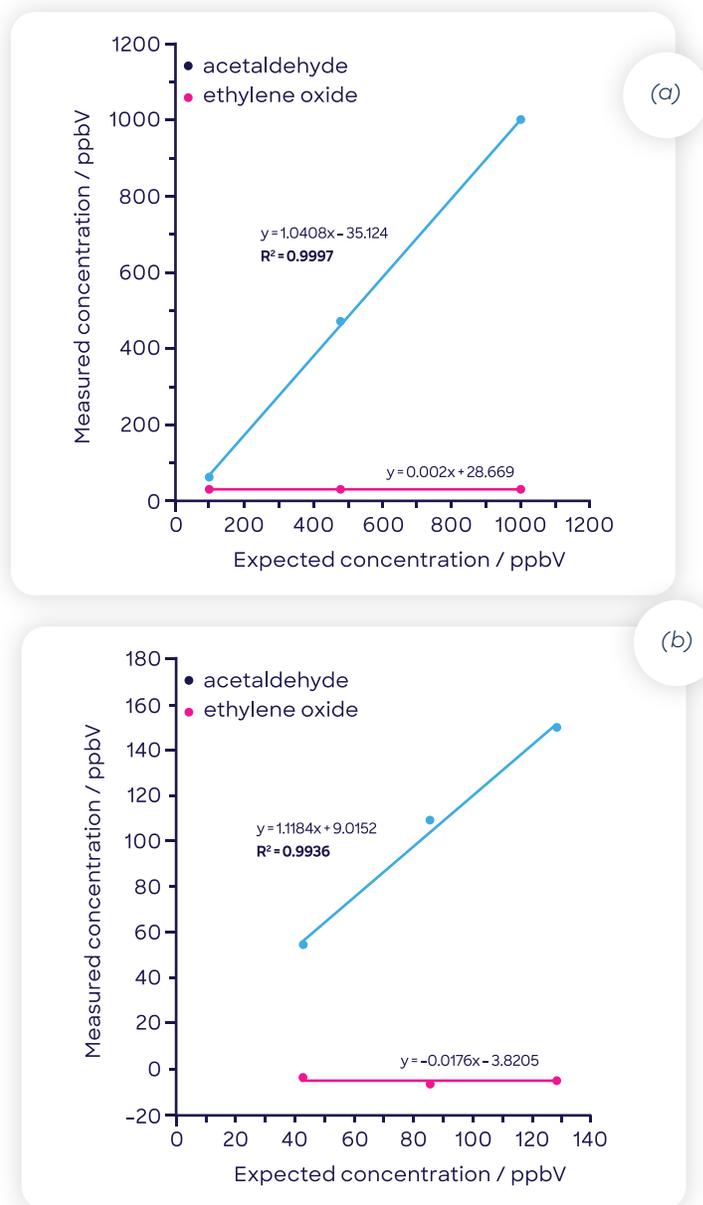
ethylene oxide than are the H₃O⁺ and O₂⁺ reagent ions, so the NO⁺ product ion (*m/z* 74) can only be practically utilized at higher ethylene oxide concentrations. Linear measurement in the gas phase is demonstrated in Figure 4.

Figure 4. Linear measurement of ethylene oxide in the gas phase using SIFT-MS.



Acetaldehyde is the major potential interferent for ethylene oxide – not dissimilar to the compendium GC-FID method (USP (2015)), despite the very different analytical approaches. Hence the SIFT-MS reaction chemistry of acetaldehyde is summarized in Table 1. Because Tween 80® samples will typically have low levels of ethylene oxide – and especially as this work aims to leverage maximum sensitivity to reduce sample size and mitigate matrix effects – a subtraction approach is utilized. The H₃O⁺ reagent ion is used to measure the combined ethylene oxide and acetaldehyde concentration and NO⁺ is used to measure the acetaldehyde concentration, enabling ethylene oxide to be determined by subtraction of the latter value from the former. Figure 5 shows that these compounds are readily separable using this approach. The signal of acetaldehyde remains negligible with increasing concentration of ethylene oxide (Figure 5(a)) and vice versa with increasing concentration of acetaldehyde (Figure 5(b)).

Figure 5. Effective discrimination and linear detection of (a) ethylene oxide and (b) acetaldehyde in samples containing both compounds.



3. Samples

The commercial Polysorbate 80 product used in this study was Tween 80® (Croda, Inc., Edison, New Jersey).

In contrast to the compendium method (USP (2013)), which uses 1 g of sample per test, the quantity of Tween 80® used here ranges from 5 to 100 mg, with water balance to give a total sample volume of 1 mL. The reduced sample volume of 1 mL was selected over the typical USP sample volume of 2.2 mL because incubation times and headspace equilibration had been optimized for the lower volume.

Results and Discussion

The limit of quantitation for SIFT-MS analysis of ethylene oxide partitioning from Tween 80® and water mixtures was determined by analyzing small quantities of Tween 80® in water. The SIFT-MS results are shown in Figure 3 and clearly show that ethylene oxide is detectable above baseline even when only 5 mg of Tween 80® is used (the signal-to-noise ratio is 5.0). Triplicate samples were analyzed at 25 mg, giving a relative standard deviation (RSD) of 4.5%, which is consistent with other headspace-SIFT-MS studies (Langford and Perkins (2021a, 2021b, 2022)).

Figure 6(a) shows the ethylene oxide response obtained using headspace-SIFT-MS as the mass of Tween 80® is varied (5 to 94 mg). Since the matrix composition is changing as the mass of Tween 80® increases, the quadratic fit suggests that there may be a matrix effect at the lower dilution levels (50 and 100 mg). Figure 6(b) shows that a linear fit (regression coefficient, R^2 , of 0.996) is obtained at higher dilutions (5 to 25 mg of Tween 80®), demonstrating that in this range the matrix effects are eliminated, and that the system is approximating water. These high dilution levels are practical for SIFT-MS, but not for GC-FID, due to the significantly higher sensitivity of the SIFT-MS technique.

Figure 6. Ethylene oxide concentrations for Tween 80® diluted in water determined using headspace-SIFT-MS analysis (a) across the full dilution range and (b) at the three highest dilution levels (where matrix effects are eliminated).

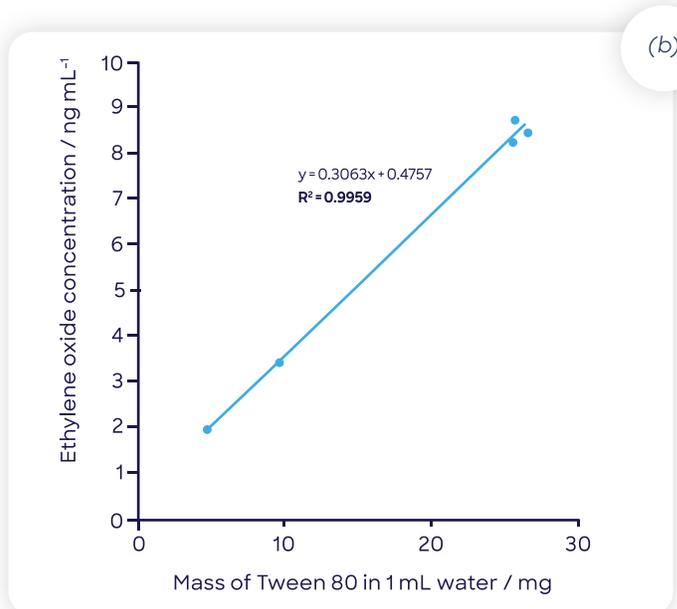
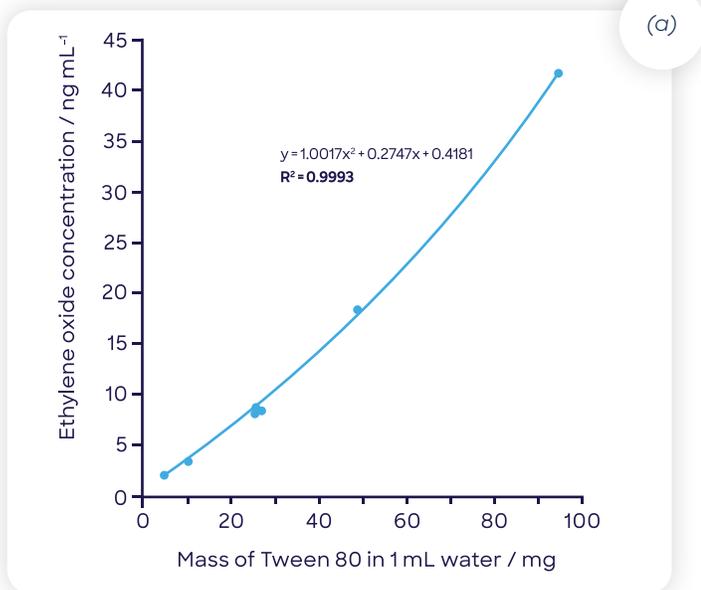
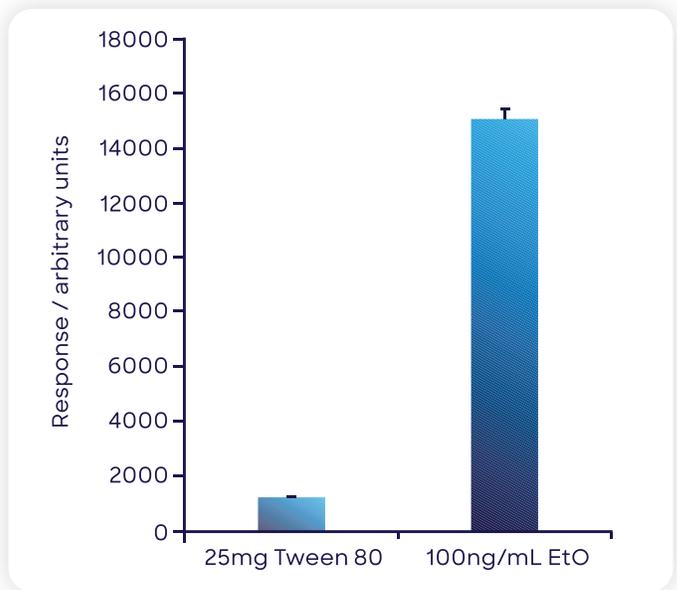


Figure 7 shows the mean of triplicate ethylene oxide measurements of the 25-mg Tween 80® sample in water and the aqueous 100-ng mL⁻¹ ethylene oxide standard. Utilizing the single-point calibration in water, 8.47 ng of ethylene oxide was obtained for the 25-mg Tween 80® sample, yielding a concentration in the Tween 80® sample of 339 ng g⁻¹ (or 0.339 ppm(w/w)). No adjustment for matrix effects was required because they were negligible at these relatively high dilution levels in water. It is postulated, therefore, that when analyzing ethylene oxide in Polysorbate 80 using headspace-SIFT-MS, only a 10-mg sample needs to be used in 1 mL of water, with calibration conducted against an external standard prepared in water. This eliminates the need to purify PEG for matrix-matching the calibration standard, when using GC-FID, greatly reducing the sample preparation for the analysis.

Figure 7. Measurement of the concentration of ethylene oxide in Tween 80® based on a single point external calibration. Both solutions were prepared in water. The small volume of Tween 80® used negated any matrix effects from PEG. The error bars represent 1 standard deviation of triplicate measurements.



Conclusions

- High-sensitivity headspace-SIFT-MS analysis detects ethylene oxide in 100-fold lower mass of sample, enabling 6-hr prep of matrix-matched standards to be eliminated.
- Headspace-SIFT-MS analysis of ethylene oxide is 9- to 14-fold faster than the compendium GC-FID method, yielding sample throughputs of up to 224 samples/day.
- Elimination of matrix-matched standards and faster analysis enables SIFT-MS to deliver the first test result over eight-fold faster than GC-FID.
- Simple sample prep.
- Simple instrument operation.
- Industry-proven technology ready for the QA/QC lab and process line.

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