

# ETHYLENE OXIDE AND ACETALDEHYDE IN POLYSORBATE 80:

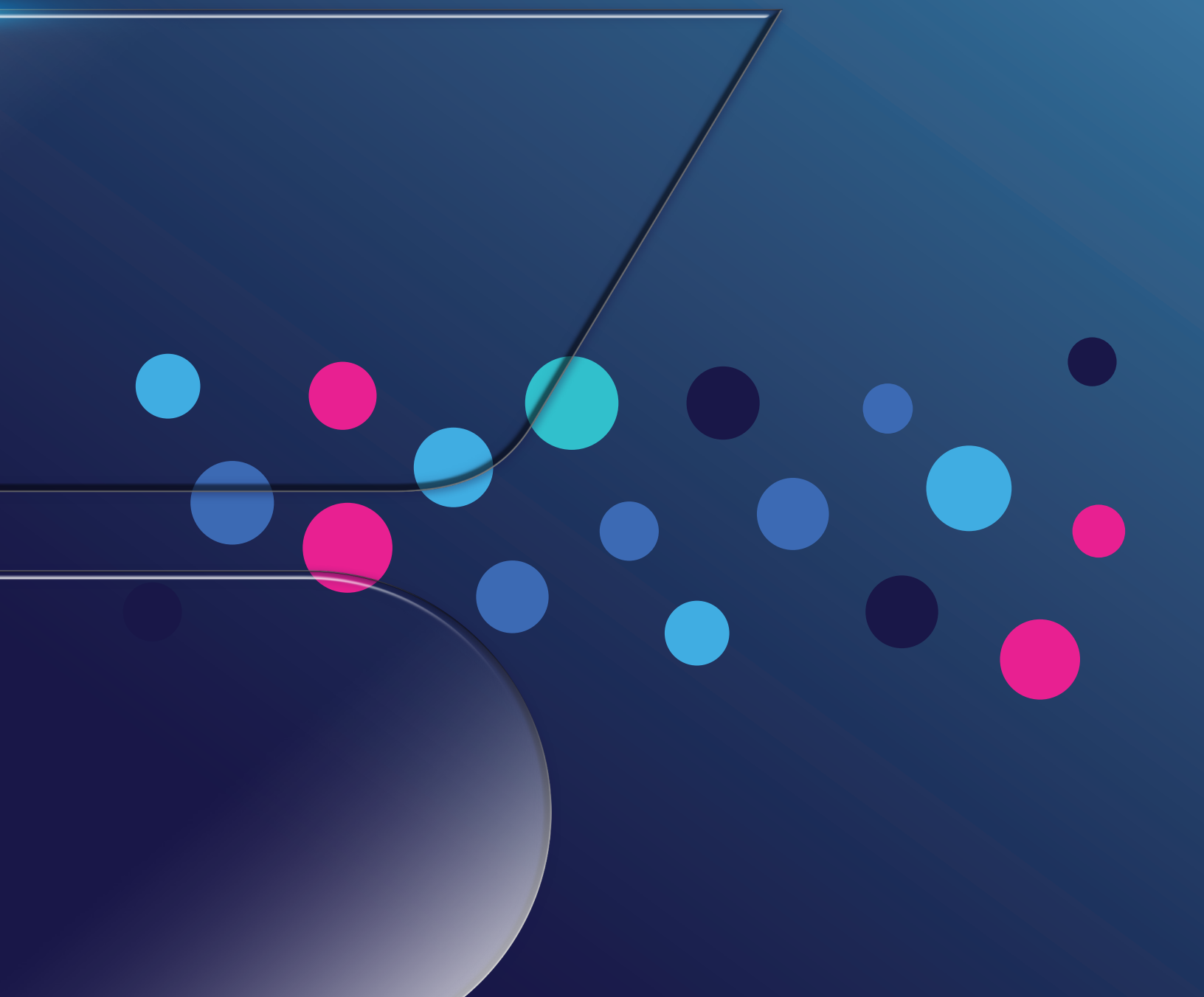
## RAPID QUANTITATIVE ANALYSIS USING SIFT-MS

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## Abstract

Selected ion flow tube mass spectrometry (SIFT-MS) accelerates ethylene oxide and acetaldehyde analysis in Polysorbate 80 excipients through elimination of very slow sample preparation and shorter run times. Building on the previous study of Silva et al. (2022), this study evaluates the postulated headspace-SIFT-MS approach for Polysorbate 80 products from multiple suppliers and evaluates ethylene oxide and acetaldehyde recoveries. Results confirm that the headspace-SIFT-MS procedure reliably quantifies these analytes without requiring purification of Polysorbate 80 for matrix-matching the calibration standard. Hence, headspace-SIFT-MS analysis of ethylene oxide and acetaldehyde can revolutionize existing workflows.

## Introduction

Conventional ethylene oxide (EtO) analysis in Polysorbate 80 products using gas chromatography with flame ionization detection (GC-FID; United States Pharmacopeia (USP) (2013,2015)) has slow time to result and slow throughput. As described in an earlier application note (Silva et al. (2022)), the significantly greater sensitivity of selected ion flow tube mass spectrometry (SIFT-MS) greatly reduces sample preparation times by eliminating matrix matching of blanks, while direct sample analysis provides significantly higher throughputs (Figure 1). Depending on the approach taken with sample prep (in parallel or on demand), headspace-SIFT-MS reports the first sample result four- to eight-fold faster than the compendium GC-FID method and has nine- to 14-fold higher daily sample throughput (Silva et al. (2022)).

In this application note, the simplified SIFT-MS analytical procedure is applied to four commercial Polysorbate 80 samples and a recovery study is conducted. It confirms the ability of headspace-SIFT-MS to rapidly quantify ethylene oxide in Polysorbate 80, including in the presence of acetaldehyde.

## Method

### 1. The SIFT-MS technique

This work utilized a Syft Tracer™ 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. (2023)) that can rapidly quantify volatile organic compounds (VOCs) down to part-per-trillion concentrations (by volume, pptV). Up to eight reagent ions ( $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$ ,  $\text{O}_2^+$ ,  $\text{O}^+$ ,  $\text{OH}^+$ ,  $\text{O}_2^-$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ; Hera et al. (2017)) obtained from a microwave discharge in air are available on Syft Tracer™ instruments. 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 ( $\text{N}_2$ ,  $\text{O}_2$  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 (Langford (2023)). The multiple reagent ions frequently remove uncertainty from isobaric overlaps in mixtures containing multiple analytes. Hence Syft Tracer™ sets the standard for sensitive and selective real-time analysis of volatile compounds.

**Figure 1.** Schematic summary of the different sample preparation approaches in the USP Polysorbate 80 monograph (USP (2015)) and the postulated headspace-SIFT-MS alternative (Silva et al. (2022)).

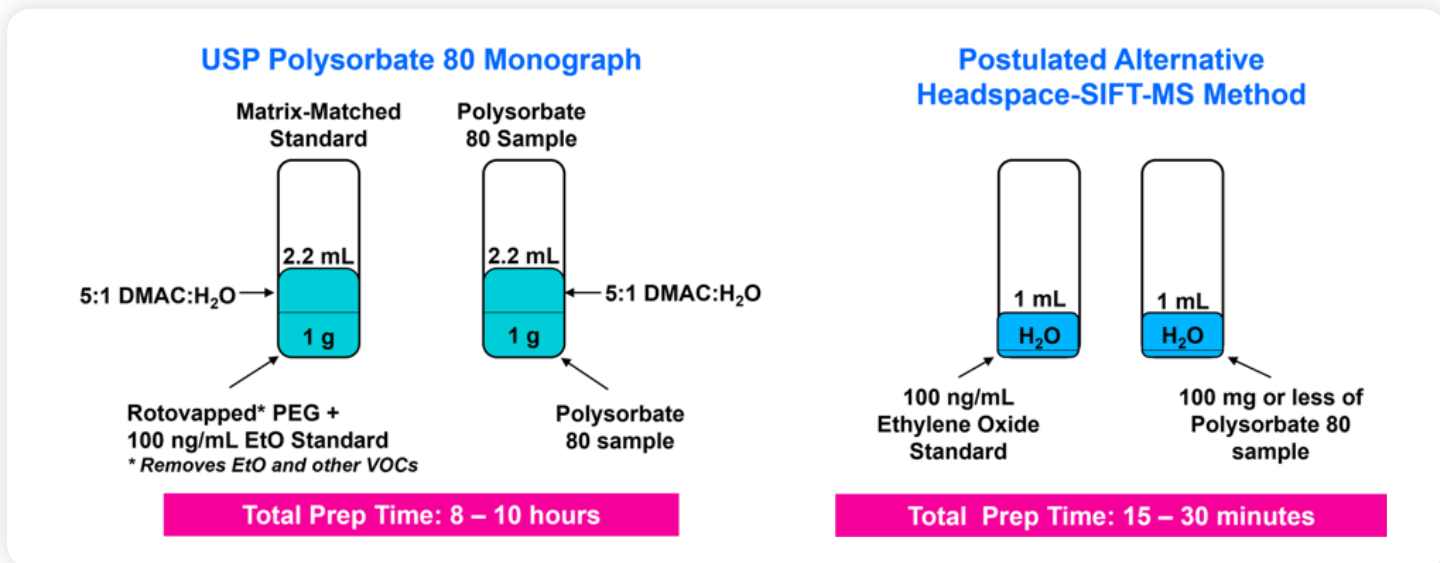
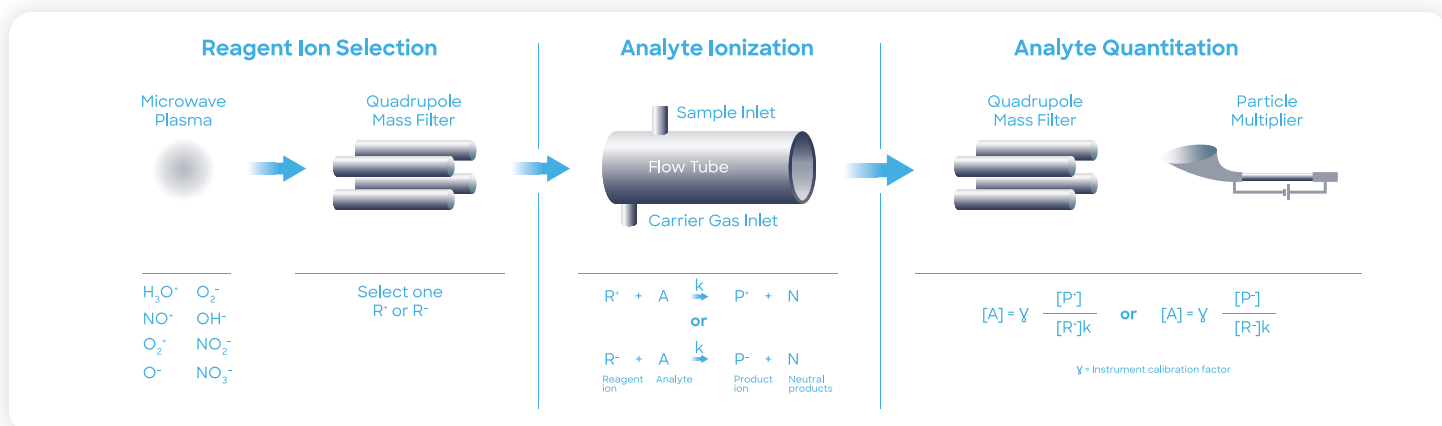


Figure 2. Schematic diagram of the SIFT-MS technique, which utilizes soft chemical-ionization for direct analysis of samples.



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 30 min. in a GERSTEL agitator. Headspace 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. The analysis time for each sample was 120 s. Note that no internal standard was utilized (Perkins and Langford (2021)).

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

SIFT-MS readily detects ethylene oxide, as demonstrated in Silva et al. (2022). Acetaldehyde is the major potential interferent for ethylene oxide – c.f. the compendium GC-FID method (USP (2015)) – and this is addressed as discussed previously (Silva et al. (2022)). Briefly, the  $\text{H}_3\text{O}^+$  reagent ion is used to measure the combined ethylene oxide and acetaldehyde concentration and  $\text{NO}^+$  is used to measure the acetaldehyde concentration, enabling ethylene oxide to be determined by subtraction of the latter value from the former.

## 3. Samples

Four commercially available samples of Polysorbate 80 were tested, as summarized in Table 1. Solutions (10% in water) were prepared, and 1 mL was placed in 20-mL sample vials. Quantitation was achieved via calibration with separate 1 ppm solutions (in water) of acetaldehyde and ethylene oxide. A combined solution (1 ppm each of acetaldehyde and ethylene oxide) was used to confirm the efficacy of the subtraction procedure.

The recovery study was conducted on the same Polysorbate 80 products. They were prepared as above but with addition of 1 ppm acetaldehyde and 1 ppm ethylene oxide spikes (both separately and together).

## Results and Discussion

Figure 3 shows the ethylene oxide and acetaldehyde

Table 1. Polysorbate 80 samples analyzed in this study.

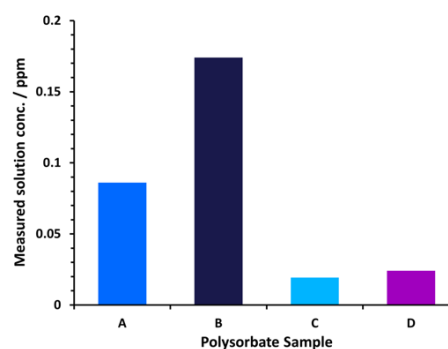
| Sample ID | Product detail                                    | Supplier                      |
|-----------|---|-------------------------------|
| A         | Tween® 80   | MP Biomedical, Ohio, USA      |
| B         | Tween® 80 for cell culture (product no. P4780)    | Sigma-Aldrich, Gillingham, UK |
| C         | Polysorbate 80 (product no. W291706)              | Sigma-Aldrich, Gillingham, UK |
| D         | Polysorbate 80 Ph. Eur. Grade (product no. 59924) | Sigma-Aldrich, Gillingham, UK |

concentrations measured in the four Polysorbate 80 products using headspace-SIFT-MS. The ethylene oxide results are all well below the 1 ppm limit defined by European and United States pharmacopeias. Acetaldehyde impurity levels are significantly higher than ethylene oxide in all samples, though the relative proportions change.

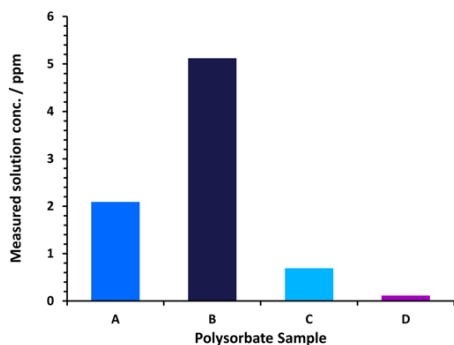
Results of the recovery study are summarized in Figure 4. These demonstrate that headspace-SIFT-MS selectively quantifies ethylene oxide. This is achieved not only in the presence of spiked acetaldehyde, but also the presence of higher acetaldehyde residues in the samples themselves (especially samples A and B, Figure 3(b)). Acetaldehyde also demonstrates good recovery when spiked into samples. The cause of the artefact when ethylene oxide was spiked into sample B needs to be investigated in future work.

Figure 3. Concentrations (ppm in solution) of (a) ethylene oxide and (b) acetaldehyde in four Polysorbate 80 samples (A to D) determined using headspace SIFT-MS.

(a)

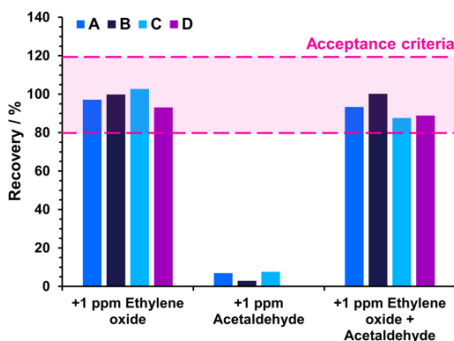


(b)

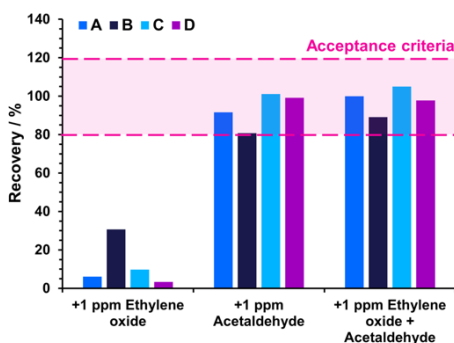


**Figure 4.** Recovery of (a) ethylene oxide and (b) acetaldehyde from the four samples (A to D) when spiked with three 1-ppm standard solutions. Acceptance criteria of 20% are shown. Ethylene oxide should not be recovered from an acetaldehyde spike.

(a)



(b)



## Conclusions

- High-sensitivity headspace-SIFT-MS analysis quantifies ethylene oxide in reduced quantities of Polysorbate 80, enabling slow 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 eight-fold faster than GC-FID.
- Simple quantitation of acetaldehyde interferent.
- Simple sample prep and instrument operation.
- Industry-proven technology ready for the QA/QC lab and process line.

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