Improvements in the Vapor-Time Profile Analysis of Explosive Odorants using Solid-Phase Microextraction

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Abstract

A modified approach for characterization of the vapor-time profile of the headspace odors of explosives was developed using solid-phase microextraction (SPME) incorporating introduction of an externally-sampled internal standard (ESIS) followed by gas chromatography/mass spectrometry (GC/MS) analysis. With this new method, reproducibility of the measurements of 2-ethyl-1-hexanol and cyclohexanone were improved compared to previous work [5,6] through the use of stable-isotope-labeled internal standards. Exposing the SPME fiber to the ESIS after sampling the target analyte proved to be advantageous, while still correcting for fiber variability and detector drift. For the analysis of high volatility compounds, incorporation of the ESIS using the SPME fiber in the retracted position minimized the subsequent competitive loss of the target analyte, allowing for much longer sampling times.

Keywords: Solid-phase microextraction (SPME); Externally sampled internal standard (ESIS); Vapor-time profile; canine training aid; explosive vapors

1. Introduction

Canines are unmatched as sensitive and portable systems for contraband detection. A critical aspect of successful canine detection relies on well-controlled initial and maintenance training protocols. For example, Kranz et al.[1, 2] recently reported that canines varied in performance when trained using different training materials. However, use of contraband materials as training materials requires care in storage and chain-of-custody. Alternative training aid materials (often termed pseudo-, mimic, or simulant training aids) contain the characteristic odorants of the hazardous materials, but in a form that is safe to handle. Ideally, alternative training aid materials should be easy to use, accessible, non-hazardous, and representative of the characteristic odor profile of

1 hazardous materials [3]. Although the use of non-explosive training aids as replacements
2 for real explosives have yet to be widely accepted by the canine detection community, the
3 development of these alternative training aids would greatly facilitate canine training.
4 Developing alternative training materials not only requires presenting the key
5 characteristic odors, but also requires knowledge about the longevity of the odor release
6 by the training aid. The vapor profiles of complex materials such as decomposing human
7 remains [4, 5] and explosive materials [1] are known to change over time, yet few studies
8 have been performed to evaluate their vapor-time profiles. As part of the development of
9 alternative training aids, the vapor-time profile of the aids must be monitored to
10 determine its functional lifetime. Minimizing uncertainty and bias in the vapor-time
11 measurement will provide the most accurate assessment of alternative training aid
12 performance.

13 One attractive approach to characterizing the headspace vapor profile of a sample is to
14 use Solid-Phase MicroExtraction (SPME) [6, 7]. However, quantitation using SPME has
15 focused primarily on determining the composition of the solid or liquid sample in
16 equilibrium with the headspace [8-11]. Recently, SPME characterization of the vapor-
17 time profile of the headspace odors of explosives was achieved through the introduction
18 of an externally-sampled internal standard (ESIS) in conjunction with gas
19 chromatography/mass spectrometry (GC/MS) analysis [12, 13]. Measurement variability
20 over time is corrected by determining the ratio of the peak area of the vapors of the
21 sampled analyte to the peak area of the sampled saturated vapors of the internal standard
22 (the A/E ratio). This approach allows the determination of a reproducible vapor-time
23 profile over at least several days [13] and quantitation in a non-equilibrium setting such as
24 in functional training aid vapor release.

25 However, choosing the sampling parameters and the proper ESIS compounds are
26 critical for quantitation with SPME-ESIS. Prior work used geometric isomers of the
27 analytes as the ESIS compounds. The isomers used had approximately similar chemical
28 properties but different retention times than the analyte of interest, specifically 1-octanol
29 for 2-ethyl-1-hexanol (2-EH) and 3,4-dinitrotoluene (3,4-DNT) for 2,4-dinitrotoluene
30 (2,4-DNT). However, this experimental approach was restricted to short sampling times
31 for the ESIS, required to minimize preferential absorption of the analyte or internal

standard determined by the limited absorptive capacity of the SPME fiber. In real world training aid performance evaluations, materials release low vapor concentrations and require long SPME sampling times. This will limit the quantitation capabilities using this experimental approach.

The aim of this study is to further optimize the SPME-ESIS approach for vapor-time determinations of functioning training aids. Optimal containers were selected to mimic canine training aid assembly and allow more reliable evaluation of alternative training aids. Another important facet of the study was to evaluate the use of stable-isotope analogs of the target analytes as the ESIS compounds. Another goal was to optimize the sampling times and protocol for the target analytes. Investigations include altering the sampling order of the analyte and the ESIS to provide more accurate measurements for vapor-time profile analysis. Also, SPME absorption with a retracted fiber was investigated for quantitation of analytes with high volatility. Analytes of interest include military and improvised explosives associated with terrorist events, specifically triacetone triperoxide (TATP), 2,4,6-trinitrotoluene (TNT), Composition C-4 (C-4), and Semtex. Characteristic odors of these military explosives include 2-EH and cyclohexanone, which have been used in previously-developed alternative training aid materials for explosives [14, 15]. We describe novel developments in SPME sampling methods employing the deuterated analogs of selected explosive odors, d_4 -cyclohexanone and d_{17} -2-ethyl-1-hexanol (d_{17} -2-EH).

2. Materials and methods

2.1 Reagents

Cyclohexanone (99 % ACS Reagent, Sigma-Aldrich St. Louis, MO) and 2-ethyl-1-hexanol (2-EH) (≥ 99.6 %, Eastman Chemical Company by Sigma-Aldrich) were chosen as representative volatiles associated with plastic explosives [15]. For TATP, the stock solution was prepared in house with $68.2 \mu\text{g g}^{-1}$ and $96.7 \mu\text{g g}^{-1}$ of TATP and ^{13}C -TATP (as the ESIS), respectively, in acetonitrile. 2,6-Dinitrotoluene (2,6-DNT) and 2,4-dinitrotoluene (2,4-DNT) (S.A.R.M., AMSMC-QAD-C(a), Picatinny, NJ) were selected as volatiles associated with military TNT using 3,4-dinitrotoulene as the internal standard. ESIS studies using isotope-labeled standards include d_{17} -2-EH (98 %, Cambridge Isotope

Labs Inc., Tewksbury, MA), ring-d₃-2,4-DNT (98 %, Cambridge Isotope Labs Inc.), and 2,2,6,6-d₄-cyclohexanone (98 %, Cambridge Isotope Labs Inc.). Comparison studies were performed with 1-octanol (99 %, Chem Service, West Chester, PA), previously used as an ESIS for 2-EH. Standards of these components were analyzed to determine the GC/MS method for best sensitivity and reproducibility. An experiment evaluated an alternative training aid (Provisional Patent no. 20140021270). Briefly, these aids consist of 5.5 g of Space Grade Encapsulant (Dow Corning 93-500, Midland, MI) polydimethylsiloxane (PDMS) cast into a 6 cm x 2 cm circular metal can. This mass provided $\approx$ 2 mm depth of PDMS. The can had twelve 2 mm holes, 6 on the top and 6 on the sides. The cast aids were then baked to remove adventitious volatile components. The training aids were vapor-infused with 2-EH odors by exposing the pre-treated PDMS polymer to the vapors from 500 μ L of 2-EH in a sealed 9 cm x 5 cm circular can for 10 days and used without further modification.

2.1.1 Calibration Solutions. Internal standards were added to the stock solutions of the odorants and diluted with acetonitrile (J.T. Baker HPLC UltraGradient Solvent, Phillipsburg, NJ) to prepare 5 or 6 point calibration curves with concentrations chosen that resulted with 1:1 ratio peak areas for the analyte and internal standard. Solutions included TATP with ¹³C-TATP as an internal standard (0.614 μ g g⁻¹ to 14.4 μ g g⁻¹ and 1.05 to 24.6 μ g g⁻¹, respectively), 2-EH and d₁₇-2-EH (0.738 μ g g⁻¹ to 19.6 μ g g⁻¹ and 0.766 μ g g⁻¹ to 19.8 μ g g⁻¹, respectively), cyclohexanone and d₄-cyclohexanone (0.978 μ g g⁻¹ to 18.9 μ g g⁻¹ and 0.513 μ g g⁻¹ to 9.89 μ g g⁻¹, respectively), and 2,4-DNT and d₃-2,4-DNT (0.916 μ g g⁻¹ to 20.1 μ g g⁻¹ and 0.926 μ g g⁻¹ to 19.6 μ g g⁻¹, respectively). The lines of best fit were based on the linear regression relationship between the mass fractions of the analytes of interest and the normalized extracted ion peak areas with reproducibility reported as relative standard deviation (RSD) values for triplicate analyses. The limit of detection (LOD) (signal-to-noise ratio (S/N) of 3) and limit of quantitation (LOQ) (S/N of 10) were estimated based on the standard deviation of the response and the slope of the calibration curve [16] and validated based on the S/N ratio observed at low mass fractions and blank samples.

2.2 Instrumentation

Experiments were conducted through injection by a Gerstel MPS2 robotic autosampler (Linthicum, MD) with controlled SPME capabilities in conjunction with an Agilent 6890 Gas Chromatograph with a Hewlett Packard 5973 Mass Selective Detector. A relatively short, non-polar DB-5 column (15 m, I.D. 0.25 mm, film 0.25 μ m, Agilent Technologies, Santa Clara, CA) was used in order to minimize the on-column decomposition of the unstable explosives-related compounds. The injection port temperature and carrier flow (helium) were varied to optimize the sensitivity (inlet temperature from 110 °C to 250 °C and carrier flow between 1 mL min⁻¹ and 2 mL min⁻¹). Increasing the injection port temperature up to 180 °C did not sacrifice the sensitivity for TATP and improved the sensitivity for the other study compounds. Increasing the flow rate did not improve the peak shape and resulted in poorer sensitivity. Quantitation studies were then continued using an injection port temperature of 180 °C and carrier gas flow of 1 mL min⁻¹. The GC method for analysis of liquid injections used a split/splitless injector with split ratio of 5:1 and a flow rate of 1 mL min⁻¹. The GC oven was programmed from an initial temperature of 50 °C (held for 3 min) to 180 °C at 10 °C min⁻¹, followed by an increase to 280 °C (held for 1 min) at 20 °C min⁻¹. The same instrumental method was used for SPME analysis except the split ratio was increased to prevent saturation of the detector.

Vapor pressure measurements utilized the autosampler to sample the headspace (HS) using a 1 mL HS syringe. The split/splitless injector was used with a flow rate of 2 mL min⁻¹ and the same temperature program. The MPS agitator tray was used to equilibrate the sample at temperatures ranging from 30 °C to 50 °C for 2 hours. Since the vapor pressure of cyclohexanone and 2-EH differ greatly, the vapor pressure measurements for cyclohexanone were performed with a split ratio of 50:1, and the 2-EH measurements were performed using a split ratio of 5:1. Measurements of vapor pressure were performed using an external calibration curve carried out using the appropriate split ratios.

The electron impact (EI) mass spectrometer was operated in scan mode in the mass range of 40 m/z to 400 m/z at an electron multiplier voltage of 2082 V or operated in the selected ion mode (SIM). The fragment ion with the largest abundance was selected for quantitation (57 m/z and 66 m/z for 2-EH and d₁₇-2-EH, respectively; 98 m/z and 102 m/z for cyclohexanone and d₄-cyclohexanone, respectively; 165 m/z and 168 m/z for 2,4-DNT and d₃-2,4-DNT, respectively; and 43 m/z and 44 m/z for TATP and ¹³C₃-TATP,

1 respectively). The source and analyzer temperatures were set at 230 °C and 150 °C,
2 respectively. The mass spectrometer was autotuned daily using perfluorotributylamine
3 (PFTBA). The Enhanced Data Analysis feature was used in conjunction with the NIST
4 Mass Spectral Search Program (NIST/EPA/NIH Mass Spectral Library, Version 2.0 d) to
5 analyze all spectra collected.

6 **2.2.1 Quantitation.** Instrumental parameters were optimized to achieve the best
7 sensitivity and reproducibility for all of the analytes of interest. Calibration curves were
8 constructed in the extracted ion mode, monitoring 43/44 m/z for TATP/ $^{13}\text{C}_3$ -TATP,
9 165/168 m/z for 2,4-DNT/ d_3 -2,4-DNT, 98/102 for cyclohexanone/ d_4 -cyclohexanone, and
10 57/66 m/z for 2-EH/ d_{17} -2-EH. Other than 2-EH, the parent compounds and their isotope-
11 labeled analogs were not baseline separated by the GC method. The figures of merit were
12 determined for the analytes of interest in the total ion chromatogram (TIC) using the scan
13 mode and selected ion mode (SIM) mode (See Supplemental Table S1). The limits of
14 detection (LOD) for the analytes ranged from 0.3 $\mu\text{g g}^{-1}$ to 0.7 $\mu\text{g g}^{-1}$ with limits of
15 quantitation (LOQ) in the range of 1.0 $\mu\text{g g}^{-1}$ to 2.4 $\mu\text{g g}^{-1}$. Greater sensitivity and
16 reproducibility are achieved upon selection of fragment ion with the greatest abundance
17 and performing analyses in the SIM mode. In comparison with the results obtained in the
18 scan mode, the SIM mode achieved $\approx 1.5\text{x}$ to $\approx 3\text{x}$ lower limits of detection (0.1 $\mu\text{g g}^{-1}$ to
19 0.6 $\mu\text{g g}^{-1}$), as well as lower LOQ values (0.5 $\mu\text{g g}^{-1}$ to 2.0 $\mu\text{g g}^{-1}$). Calibration curves
20 obtained using deuterated internal standards resulted in improved LOD, LOQ and
21 reproducibility, compared to those using analytes with baseline-separated analogs.

22 **2.3 SPME analysis**

23 SPME experiments were performed using PDMS-coated non-polar silica fibers (100 μm
24 non-bonded, 23 gauge, Sigma-Aldrich) conditioned at 250 °C for 0.5 h before use.
25 Samples were evaluated in ≈ 20 mL headspace, glass autosampler vials with magnet-
26 compatible, crimp-top septa caps (Gerstel). SPME/ESIS experiments primarily
27 investigated using 2-EH and cyclohexanone and their respective ESIS (1-octanol, d_{17} -2-
28 EH, and cyclohexanone). SPME analysis of 2-EH and the ESIS (1-octanol or d_{17} -2-EH)
29 were prepared by injecting very low quantities (1 μL) of the pure analyte into a vial using
30 a gas-tight syringe, crimp-sealing, and allowing the headspace to reach equilibrium for a
31 minimum of 2 hours. ESIS compounds were prepared by placing larger volumes of the

neat standard (>100 μL) in 20 mL headspace vials. The SPME fiber was then exposed using varying sampling times (3 s to 5 min) either symmetrically (same sampling time for the ESIS and the sample) or asymmetrically (different sampling times for the ESIS and the sample) [13]. Similar sampling conditions were conducted for cyclohexanone with d_4 -cyclohexanone as the ESIS.

One-gallon (3.78 L) paint cans were also used for headspace analysis to simulate a typical assembly in which canine training aids are placed. A purpose-built tray system was developed to affix the gallon can and allow for automatic SPME sampling using the Gerstel autosampler. Gallon metal cans (The Cary Company, Addison, IL) were prepared for sampling by drilling a hole (≈ 5 mm in diameter) in the middle of the can lid. The gallon cans were then rinsed twice with ≈ 100 mL of pentane (J. T. Baker, Baker analyzed HPLC solvent, Phillipsburg, NJ) and baked in an oven at 180°C for 2 hours. The drilled hole in the can was sealed with a sleeve stopper septa (2.2 mm bottom I.D. $\times$ 4.5 mm O.D., Sigma Aldrich) which was pre-pierced several times before performing SPME experiments. Static (closed container) and dynamic (open container) SPME extractions were performed directly above the opening of the gallon can. Gallons cans were reused by repeating the cleaning process. Background SPME GC/MS analysis validated the success of the cleaning process.

2.4 Deuterated analog vapor pressure estimations

The vapor pressures of the deuterated ESIS compounds (d_4 -cyclohexanone and d_{17} -2-EH) were determined for comparison to their non-deuterated analogs. Vapor pressure measurements were performed by adding sufficient analyte (100 μL) in 10 mL glass autosampler vials (Gerstel) to saturate the headspace. The vials were sealed and crimped with crimp-top septa caps and allowed to equilibrate for 2 hours. Vapor pressures (P) were calculated assuming that the vapors of odorants obeyed the ideal gas law [17]:

$$P = \frac{mRT}{MV} \quad (1)$$

where m is the mass of the analyte determined through an external calibration, R is the gas constant ($8.314 \text{ L kPa mol}^{-1} \text{ K}^{-1}$), T is temperature in Kelvin, V is the volume of the headspace sample injected into the GC/MS (100 μL for cyclohexanone and d_4 -

cyclohexanone; 500 μL for 2-EH and d_{17} -2-EH), and M is the molar mass of the chemical compound (cyclohexanone = 98 g mol^{-1} , d_4 -cyclohexanone = 102 g mol^{-1} , 2-EH = 130 g mol^{-1} , d_{17} -2-EH = 147 g mol^{-1}).

3. Results and Discussion

3.1 Sampling container investigations

Sufficient cyclohexanone was spiked into sealed containers to minimally saturate the headspace of the container. The concentrated vapors were monitored over a period of 24 h in which the A/E ratio (peak area ratio of the analyte to the ESIS) was calculated for symmetric sampling of 3 s fiber exposure to the analyte (cyclohexanone) and saturated vapors of the ESIS (d_4 -cyclohexanone) from a headspace glass vial with excess amount of d_4 -cyclohexanone ($>100 \mu\text{L}$). Headspace equilibrium in the 20 mL headspace glass vials with crimp tops was achieved in $\approx 1 \text{ h}$ for cyclohexanone; however, a decrease in signal was observed for longer equilibration times. Extractions of the PTFE/silicone septa showed absorption of the analyte into the septa. To alleviate this loss of analyte, aluminum-faced silicone septa (Sigma-Aldrich) were investigated. However, the calculated A/E ratios also decreased after 3 h, as shown in Figure 1. Possible adsorption of the analyte to the glass surface of the vials [18] was minimized by performing the experiment at elevated temperatures (50°C). However, the calculated A/E ratios were found to decrease at a faster rate. Another possible explanation for the decrease in signal could be coring of the septa due to the multiple sampling of the same analyte over a period of time. The SPME fiber needle has a gauge of 23 without a tapered end which might damage the surface of the septa. This would allow for greater loss of the analyte from the sealed container after repeated SPME sampling. Although increasing the temperature might reduce adsorption on the glass surface, higher temperature could also result in faster diffusion of the analyte into the septum or exiting into the atmosphere through the cored septum.

A similar cyclohexanone sampling study used a one-gallon metal can to minimize loss of analyte vapors. Gallon cans were chosen in order to simulate field canine training techniques and monitor the headspace profile of our alternative training aids over time.

To saturate the headspace of the gallon cans, addition of 200 μL of cyclohexanone was required to provide the same 0.05 $\mu\text{L mL}^{-1}$ volume fraction as used with the 20 mL glass vial. The A/E ratios were calculated from symmetrical 3 s SPME sampling of the analyte and 3 s sampling of saturated vapors of the ESIS (100 μL in 20 mL headspace vial), shown in Figure 1. Headspace concentration of cyclohexanone reached equilibrium after ≈ 3 h, with statistically negligible loss of analyte from the headspace for the 24 h of the study. In conclusion, the use of the gallon cans to simulate the vapors emitted by canine training aid materials resulted in a more representative sample of the headspace of the with minimal loss of the vapors. This set-up was used for vapor-time profile monitoring.

3.2 Investigations in selecting the proper ESIS compound

Selection of the ESIS is based on the following criteria: (1) the ESIS should have similar analytical behavior and chemical properties (e.g. vapor pressure) as the compound of interest, (2) the ESIS must not be present in the headspace of samples or react with the sample, and (3) the ESIS can be selectively detected from the target compounds. Previously, 1-octanol and 3,4-DNT were used as the ESIS for 2-EH and 2,4-DNT, respectively [12, 13]. Investigations were performed to observe the relative extraction properties for the analytes and their respective ESIS at varying sampling times. Mixtures of the ESIS (1-octanol and d_{17} -2-EH) and analyte (2-EH) were prepared in different ratios by first sealing the headspace vial and spiking the vials with the appropriate ratios of the analyte or ESIS using a gas-tight syringe. After more than 2 h of headspace equilibrium time, the PDMS SPME fiber was exposed to the headspace of the vial for varying sampling times, and the signal was monitored for the corresponding analytes. Figure 2 displays 1:4, 1:1, and 4:1 analyte/ESIS mixtures of 2-EH and d_{17} -2-EH that correspond to peak area ratios of 0.25 ($\pm 4\%$), 0.96 ($\pm 3\%$), and 4.0 ($\pm 4\%$), respectively. Increased sampling times for the 2-EH and d_{17} -2-EH mixtures resulted with fairly constant peak areas ratios and reproducible results with RSD values of $< 4\%$.

Peak area ratios obtained for 1:4, 1:1, and 4:1 2-EH/1-octanol mixtures were 1.2 ($\pm 5\%$), 4.0 ($\pm 7\%$), 16 ($\pm 4\%$), respectively. Extractions beyond 60 s for mixtures of the analyte and ESIS were observed to deviate from a constant peak area ratio (Figure 2), particularly for isomeric compounds with different distribution constants. This could be due to saturation of the SPME fiber. Upon saturation, the extraction of the analytes onto

the SPME fiber is based on the distribution coefficients for the analyte between SPME fiber and air (K_{fg}). The K_{fg} can be estimated based on their retention time in GC when using a relatively non-polar column [19]. Using geometric analogs as the ESIS, such as 1-octanol for 2-EH, resulted in different diffusion rates of the analyte and ESIS. The peak area of 2-EH was observed to be $\approx 4x$ greater than 1-octanol in pre-equilibrium extractions. Equilibrium extractions (< 60 s) resulted in greater partitioning of the 1-octanol due to its higher partition coefficient, resulting in lower peak area ratios, as observed in Figure 2. Deuterated analogs have similar retention times as their non-deuterated analogs, thus the relative amount of the compounds absorbed is based on the relative concentration of the analytes in the headspace. Deuterated analogs were further investigated to determine suitability as the ESIS compound based on other physical properties.

3.2.1 Vapor pressure measurements for deuterated internal standards. Vapor pressure measurements would allow us to determine the behavior of the analyte in the headspace and accurately determine vapor concentrations [13]. Previous vapor concentration studies were performed using the reported vapor pressures of the analyte and the analog used as the ESIS [13]. In this study, vapor pressures were calculated for cyclohexanone, d_4 -cyclohexanone, 2-EH, and d_{17} -2-EH. The plot of the vapor pressure with respect to time was used to determine the Clapeyron equations over the temperature range of 15 °C to 50 °C for cyclohexanone and d_4 -cyclohexanone, as shown in Figure 3. The dependence of vapor pressure and temperature can be expressed as: $\ln P(\text{kPa}) = -5823/T(\text{K}) + 25.485$ and $\ln P(\text{kPa}) = -4961.4/T(\text{K}) + 22.603$ for cyclohexanone and d_4 -cyclohexanone, respectively; $\ln P(\text{Pa}) = -10111/T(\text{K}) + 33.339$ and $\ln P(\text{Pa}) = -9809.8/T(\text{K}) + 32.302$ for 2-EH and d_{17} -2-EH, respectively. The slopes of the equation can be used to calculate the heats of vaporization, determined to be $48.4 \pm 5.6 \text{ kJ mol}^{-1}$ and $41.2 \pm 5.6 \text{ kJ mol}^{-1}$ for cyclohexanone and d_4 -cyclohexanone, respectively. These agree well with the reported value for cyclohexanone ($\Delta_{\text{vap}}H^\circ = 44 \pm 4 \text{ kJ mol}^{-1}$ [20]). The heats of vaporization calculated for 2-EH and d_{17} -2-EH are $84.0 \pm 3.3 \text{ kJ mol}^{-1}$ and $81.5 \pm 4.5 \text{ kJ mol}^{-1}$, respectively. These determined values of $\Delta_{\text{vap}}H^\circ$ are similar to each other but are somewhat higher than the reported value for 2-EH ($\Delta_{\text{vap}}H^\circ = 68.5 \pm 0.2 \text{ kJ mol}^{-1}$ [21]). Future studies would be necessary to determine the discrepancies between the reported

1 and literature values. The vapor pressures of the targeted deuterated compounds were
2 determined to be similar to their non-deuterated analogs which may simplify quantitation
3 of the analyte, previously calculated based on the relative response factor [13].

4 **3.3 Determination of the optimal sampling method of the ESIS and analyte**

5 **3.3.1 Choice of ESIS Compound.** A series of experiments evaluated whether
6 substituting a stable-isotope-labeled compound for a geometric isomer as the ESIS
7 improved reproducibility. 2-EH was used as a model compound comparing d₁₇-2-EH and
8 1-octanol as the ESIS (see Supplemental Information). Supplemental Table S2 provides a
9 comparison of the A/E ratios as a function of symmetrical sampling time of the vapor-
10 saturated analyte and internal standard. For sampling times between 10 s and 45 s,
11 consistent A/E values and low uncertainties were found for both ESIS compounds.
12 Sampling times shorter than 10 s resulted in A/E ratios with high uncertainties for the
13 geometric isomer ESIS compounds, most likely due to sampling time error. Longer
14 sampling times resulted in increasing A/E ratios, presumably due to displacement of the
15 ESIS by the analyte as the fiber coating became saturated. These studies showed
16 sampling times greater than 10 s of the ESIS is necessary to reduce sampling error
17 regardless of whether one uses deuterated or geometric isomers under these conditions.

18 **3.3.2 Comparing the sampling order of the ESIS and analyte.** Considering the limited
19 absorptive capacity of a SPME fiber, the introduction order of the ESIS and analyte into
20 the SPME fiber was investigated using d₁₇-2-EH and 2-EH. Comparisons were performed
21 using symmetric and asymmetric analysis of the ESIS and analyte. Asymmetric analysis
22 [13] is used in vapor-time profile studies where low concentrations of the analyte are
23 monitored. A short sampling time of the concentrated vapors of the ESIS is required to
24 prevent saturation of the SPME fiber absorptive capacity. This approach is particularly
25 advantageous in cases where long sampling times of the sample are needed due to the low
26 vapor concentration of the target analyte.

27 Symmetrically sampling the saturated vapors of the ESIS before (pre-ESIS) and after
28 (post-ESIS) sampling concentrated vapors of the analyte resulted in constant A/E ratio
29 until saturation limit of the fiber (>30 s sampling time) is reached. Similar to previous
30 studies (Table S2), the A/E ratio then deviates from a constant ratio in which pre-ESIS
31 analyses show an increase in the A/E ratio due to displacement of the ESIS from the

1 SPME fiber with analyte in the headspace and deviations to lower A/E ratio of post-ESIS
2 analyses with d₁₇-2-EH displacing 2-EH in the SPME fiber (see Figure S1).

3 Asymmetrical sampling of the ESIS before and after sampling the analyte was also
4 investigated. Saturated vapors of the ESIS (d₁₇-2-EH) were sampled for 3 s either before
5 or after sampling the vial containing saturated vapors of the analyte (2-EH), sampled for 3
6 s to 10 min. Increased A/E ratios with increased sampling were observed, as shown in
7 Figure 4. However, pre-ESIS analysis showed a linear trend in the A/E ratio with
8 increased variability whereas post-ESIS analysis displays a SPME sampling time sorption
9 curve with good reproducibility. Thus, for asymmetrical sampling of analytes with low
10 vapors requiring long extraction times (e.g. dynamic sampling of explosives and
11 alternative training aids where vapor concentrations are low), introduction the ESIS after
12 headspace sampling of the analyte prevents loss of the ESIS into the atmosphere of the
13 autosampler or headspace of the analyte container and results in a more predictable and
14 reproducible headspace profile. For analytes with low vapor concentration that require
15 long sampling times (>60 s), the A/E ratio using an ESIS sampled after the analyte
16 corrects for fiber variability and instrument drift [12], effectively recalibrating the fiber
17 for each measurement.

18 Real training aid sampling scenarios require long sampling of the analyte due to the
19 lower vapor concentration of the analyte by dynamic loss from the open containers
20 compared to the saturated vapors of the ESIS. The initial pre-ESIS technique introduces
21 the SPME fiber with concentrated vapors of the ESIS into the sample vial. Under static
22 sampling conditions, the ESIS partitions into the headspace of the sample vial kinetically,
23 thereby contaminating the sample vial. As a result, loss of the ESIS signal might also
24 result in overcompensation of the A/E measurements of the analyte over time. ESIS-
25 SPME analyses of blank containers were studied to observe the relative loss in signal
26 from the ESIS. Symmetric pre-ESIS sampling in closed containers resulted in little loss
27 of the ESIS signal for short sampling times (<30 s). The ESIS, however, was detected in
28 the blank vial after pre-ESIS analysis using longer sampling times. The ESIS diffuses
29 from the SPME fiber at a faster rate when performing dynamic studies in open containers
30 (Figure 5a). Signal loss was evident after 10 s sampling and ESIS signal loss of > 99 %
31 after 5 min sampling. In addition, asymmetric sampling by pre-ESIS (3 s sampling of the

ESIS followed by 5 s to 300 s sampling of a blank container) resulted in loss of the ESIS signal up to 53 % during static extractions and ESIS signal loss of up to 97 % during dynamic extractions (Figure 5b). Post-ESIS asymmetric extractions resulted in negligible loss of the ESIS signal. In addition, extraction of the ESIS vial showed negligible detection of analyte. Although potential loss of the analyte might result from extraction prior to the ESIS, the ESIS extraction is obtained using short sampling times under static conditions which was observed to result in negligible loss of the analyte from the SPME fiber or into the ESIS vial.

3.3.3 Fiber-retracted SPME sampling of the ESIS. One of the goals for using the ESIS is to improve the reproducibility of SPME measurements. Possible loss of the analyte from post-ESIS sampling and elevated variability from short sampling times of the ESIS limited the reproducibility of the results. To address this issue, post-ESIS sampling was performed using sampling with the SPME fiber retracted into the sampling needle. This protocol introduces the ESIS into the fiber coating limited by diffusion through the cross section of the orifice of the needle. This roughly represents a reduction in exposed fiber surface area of ≈ 100 . This estimation is based on the cross sectional face area of a 100 μm PDMS coated SPME fiber, diffusion of the ESIS onto the SPME fiber being limited to $\approx 0.09 \text{ mm}^2$ of the inside diameter of the 23 gauge needle, in comparison to a fully-exposed SPME fiber (9.5 mm^2 for 1 cm SPME fiber). Of course, absorptive accumulation of the ESIS would be expected to occur on the any static air gap between the opening and retracted fiber coating as well as end-face of the fiber.

This sampling method preserves the quantity of adsorbed analyte by not exposing the SPME fiber fully to the high concentration of the ESIS. It also provides an opportunity for longer, more reliable sampling times (min versus s) of the ESIS vapor without fiber or detector saturation. The autosampler was programmed to first extend the fiber 1 cm when sampling the analyte container, retract it, and then the fiber was exposed to the ESIS container without extension. The reproducibility of the ESIS was monitored by fiber-retracted SPME sampling of the ESIS from 3 s to 10 min (see Table S3). Short sampling times ($< 30 \text{ s}$) resulted in higher RSD values (14 % to 23 %) due to small peak areas that were close to the instrumental detection limit. ESIS sampling of $\geq 30 \text{ s}$ achieved more reproducible signals for the ESIS without oversaturating the detector.

Studies were performed to determine loss of the analyte with extended sampling time of the ESIS when the fiber is not extended. This was performed by asymmetric static sampling the analyte for 10 s followed by 1 min to 10 min fiber-retracted sampling of the ESIS. The same protocol was used for static sampling of 2-EH for 10 s followed by fiber-retracted sampling of a blank vial for up to 10 min to monitor the relative loss from this delay in detection. Results (Figure 6) showed negligible loss in signal of the analyte with increased sampling of the ESIS. In fact, increased sampling time improved the reproducibility of the ESIS (RSD for the peak areas < 2 %) with high reproducibility of the analyte (RSD values for the peak areas ranging from 0.2 % to 6 %).

3.3.4 Vapor-time profile of alternative training aids. The fiber-retracted ESIS method is suitable to monitor the vapor-time profile of the alternative training aids to evaluate their performance and useful lifetime. To test this method, the vapor-time profile of alternative training aid materials was monitored in an open, dynamic system to mimic the vapors released for canine training. PDMS alternative training aid material, vapor-infused with excess amount of 2-EH was monitored for a period of 24 h, performing SPME extractions for 5 min directly above the open metal can containing the training aid material followed by 1 min fiber-retracted ESIS extraction. For comparison, 100 μ L of 2-EH was directly spiked into the open metal can, and the vapor-profile was monitored using the same extraction method. The vapor profiles for the headspace of the 2-EH from these two sources are shown in Figure 7. The PDMS alternative training aid material was observed to have a constant vapor concentration in the open container over a period of 24 h. In contrast, spiking 100 μ L of the 2-EH standard into the open container resulted in varying 2-EH vapor concentration. Initially, high vapor concentrations were detected, decreasing at a rapid rate within the first 5 h. The vapor concentration of 2-EH stabilizes for ~ 4 h and then decreased at a steady rate as it is exhausted by evaporation, becoming completely depleted in detectable vapors at times > 17 h. The PDMS alternative training aid infused with volatile components (e.g. 2-EH) appears to be a promising approach to providing uniform concentration of a volatile component in a realistic setting over a period of 24 h. Future studies will evaluate their performance with longer sampling times.

4. Conclusion

This study successfully optimized approaches for vapor-time profile analysis of the volatile chemical signatures associated with military explosives (2-ethyl-hexanol and cyclohexanone). The use of the gallon cans to simulate the vapors of canine training aid materials permits the analysis of training aid materials without further modification, resulting with a representative sampling of the headspace of the training aid materials. This experimental setup delivers accurate SPME headspace extractions with minimal loss of the analyte in the headspace.

The improved ESIS-SPME method minimizes the uncertainty and bias in the measurement and provides the most accurate assessment of alternative training aid performance. By using a stable isotope labeled analyte as the ESIS, the internal standard and analyte behave very similarly in the gas phase and are extracted in the SPME fiber at a constant ratio. The vapor pressure measurements determined that the vapor pressures of the analyte and their deuterated isotope are very similar in agreement with the reported values, which will simplify the quantitation of vapor concentrations of alternative training aids. Reproducible results were achieved by sampling the analyte first, followed by fiber-retracted sampling the concentrated vapors of the ESIS. This allows longer sampling times of the ESIS to improve reproducibility without saturating the SPME fiber and minimizing loss of the analyte. The fiber retracted method was chosen to prevent loss of the analyte from interacting with the concentrated vapors of the ESIS. Exposure of the fiber at different lengths could also be used for analytes with an ESIS that requires longer extraction times (e.g. 2,4-DNT). Further studies will be necessary for quantitation of analytes absorbed in the SPME fiber for vapor-time profile measurements of alternative training aid materials.

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1 and Technology, nor is it intended to imply that the materials or equipment identified are
2 necessarily the best available for the purpose.

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Tables and Figures

Figure 1. Headspace vapor-time profile of saturated cyclohexanone vapors (1 μL in 20 mL glass vial, 200 μL in gallon can). A/E ratios of cyclohexanone/ d_4 -cyclohexanone in ($\blacklozenge$) metal gallon can and ($\square$) 20 mL headspace glass vials for a period of 24 h ($n = 3$). Error bars represent standard deviations of the mean.

Figure 2. Peak area ratio for 2-EH/d₁₇-2-EH and 2-EH/1-octanol mixtures of 1:4, 1:1 and 4:1 analyte:ESIS mixture ratios (extracting ion peaks at 57/66 m/z and 57/56 m/z for 2-EH/d₁₇-2-EH and 2-EH/1-octanol, respectively) (n=3). Error bars represent standard deviations of the mean.

Figure 3. Clausius Clapeyron diagram for cyclohexanone /d₄-cyclohexanone in contrast with reported literature values for cyclohexanone [22][23] (n=3). Error bars represent standard deviations of the mean.

Figure 4. A/E ratios for asymmetrical sampling of the ESIS (3s sampling of 5 μ L d_{17} -2-EH) before ($\square$) and after ($\blacklozenge$) sampling the analyte (5 μ L 2-EH) at varying fiber exposure times ($n=3$). Error bars represent the standard deviation of the mean.

Figure 5. (a) Response of the pre-ESIS by symmetrically sampling the analyte (blank vial) with various sampling times ($n=3$, error bars denote standard deviations of the mean); **(b)** Average response of the pre-ESIS and post-ESIS by asymmetrical sampling of the ESIS (3 s) and analyte (5 s to 300 s) ($n=3$). A spline fit curve was used to indicate the general trend but does not imply a specific mathematical relationship.

Figure 6. Peak areas of 2-EH and the ESIS (d_{17} -2-EH) after 10 s sampling of 2-EH followed by fiber-retracted sampling of the ESIS for 1 min to 10 min ($n=3$). Error bars denote the standard deviations of the mean.

Figure 7. Vapor-profile study of 2-EH infused PDMS alternative training aid material and 100 µL 2-EH using 2 different SPME fibers (denoted by using different symbols: □ for Fiber 1 and ◇ for Fiber 2). A spline fit curve was used to indicate the general trend but does not imply a specific mathematical relationship.