Evaluation of the Long-Term Storage Stability of the Cyanide Antidote: Dimethyl Trisulfide and Degradation Product Identification

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**ABSTRACT:** This study reports the long-term storage stability of a formulation of the cyanide (CN) antidote dimethyl trisulfide (DMTS). The F3-formulated DMTS was stored in glass ampules at 4, 22, and 37 °C. Over a period of one year, nine ampules (n = 3 at each temperature) were analyzed by high-performance liquid chromatography (HPLC)−UV/vis at daily time intervals in the first week, weekly time intervals in the first month, and monthly thereafter for a period of one year to determine the DMTS content. No measurable loss of DMTS was found at 4 and 22 °C, and good stability was noted up to five months for samples stored at 37 °C. At 37 °C, a 10% (M/M) decrease of DMTS was discovered at the sixth month and only 30% (M/M) of DMTS remained by the end of the study; discoloration of the formulation and the growth of new peaks in the HPLC chromatogram were also observed. To identify the unknown peaks at 37 °C, controlled oxidation studies were performed on DMTS using two strong oxidizing agents: meta-chloroperoxybenzoic acid (mCPBA) and hydrogen peroxide (H₂O₂). Dimethyl tetrasulfide and dimethyl pentasulfide were observed as products using both of the oxidizing agents. Dimethyl disulfide was also observed as a product of degradation, which was further oxidized to S-methyl methanethiosulfonate only when mCPBA was used. HPLC−UV/vis and gas chromatography−mass spectrometry/solid phase microextraction analysis revealed good agreement between the degradation products of the stability study at 37 °C and those of disproportionation reactions. Furthermore, at 4 and 22 °C, chromatograms were remarkably stable over the one-year study period, indicating that the F3-formulated DMTS shows excellent long-term storage stability at T ≤ 22 °C.

**INTRODUCTION**

The word “cyanide” (CN) originated from the Greek word kyanos, meaning dark blue. CN is pervasive in nature. CN is used in electroplating, in gold and silver mining, and as a nickel aluminum battery constituent.¹,² CN is a very toxic agent, which prevents cells from utilizing oxygen. Possible intoxication routes include inhalation, dermal absorption, ingestion, or other parenteral administration.³ Exposure to CN may cause severe illness or death. When CN enters the mitochondria of living cells, it binds to the terminal enzyme of the electron transport chain, cytochrome c oxidase, resulting in acute cellular hypoxia which prevents the production of ATP. At high enough doses, this results in cell death.⁴ Depending on the pH, CN can be present as the molecular form, hydrogen cyanide (HCN), or as an anion (CN⁻). HCN is a weak acid with a pKₐ of 9.2; therefore, HCN is the dominant form in the human body at pH 7.4. Critically, HCN can easily penetrate through the cellular and subcellular membranes. High doses of CN (>5× LD₅₀) are capable of producing more adverse responses. For example, induction of pulmonary and coronary arteriolar vasoconstriction can cause pulmonary edema or cardiogenic shock.⁵ Lower doses of CN result in dizziness, headache, vomiting, and nausea due to the inhibition of cellular enzymes.

Two important categories of CN antidotes are the CN scavengers and the CN detoxifiers. The scavenger-type antidotes form stable complexes with CN, such as cyanomethemoglobin or cyan-covalent compounds. Amyl nitrite, sodium nitrite, and 4-dimethylaminophenol are examples of methemoglobin formers. In the United States, Nithiodote and Cyanokit are the most widely used CN antidotes. Nithiodote...
contains a combination of sodium nitrite and sodium thiosulfate (TS), while the active component of Cyanokit is hydroxocobalamin. Both Nithiodote and Cyanokit have limitations, such as the requirement of intravenous administration, which raises a practical concern especially for mass casualty scenarios involving a large number of victims. In addition, although TS by itself can be identified as a CN detoxifier by acting as a sulfur donor, it has a relatively low sulfur donor efficacy, which is not ideal for CN conversion in the absence of the sulfurtransferase enzyme: rhodanese.

Dimethyl trisulfide (DMTS) is proposed to be an effective CN antidote that can be administered through an intramuscular injection. DMTS is a member of the dialkyl polysulfides, and it is found in garlic in high concentrations. It is also used as a flavor enhancer in the food industry. DMTS is naturally formed as a lipophilic decomposition product from allicin (the molecule contributes to the characteristic garlic odor) with the catalysis of the alliinase enzyme in garlic. Dialkyl polysulfides are shown to be thermally unstable, especially at higher temperatures, and will undergo disproportionation reactions.

A previous study shows that DMTS donates a sulfur atom to CN more efficiently than TS. The middle sulfur atom of DMTS is proposed to bind to CN. The DMTS molecule and its formulation is currently patented as a CN antidote. Before the dialkyl polysulfide DMTS can be applied in clinical settings, further investigations of its pharmacokinetics and stability are needed. In this study, high-performance liquid chromatography–UV/vis detection (HPLC–UV/vis) and gas chromatography–mass spectrometry/solid phase microextraction (GC–MS/SPME) analyses were utilized to evaluate the long-term stability of DMTS at different temperatures and the identities of decomposition products observed at the highest storage temperature.

## RESULTS AND DISCUSSION

### Storage Methods of DMTS

Storage methods are one of the contributing factors to the stability of a drug during the drug development process. Kiss et al. reported the effects of three different storage methods of the earlier developed DMTS formulation (F2-formulated DMTS), which included the single crimp-sealed method, the double crimp-sealed method, and the hermetically fire-sealed glass ampule method. With the single crimp-sealed method, the F2-formulated DMTS was stable for only 2 days and about 60% (M/M) of the original DMTS content was lost after 1 week. The F2-formulated DMTS was slightly more stable when stored using the double crimp-sealed method, with 65% (M/M) of the original DMTS content still detected after 2 weeks. Among the three methods, the hermetically fire-sealed glass ampule demonstrated the best stability in which the DMTS content had no significant difference after a month of storage. Therefore, the fire-sealed glass ampule method was used in this study to determine the long-term storage stability of the enhanced F3-formulated DMTS. In the preliminary study, the impact of high heat (1900–2000 °C) generated from the flame during sealing was assessed. Flame was only applied to the edge of the ampule in order to avoid any direct heating of the glass contacting the F3-formulated DMTS. However, the DMTS concentrations were determined before and right after (n = 3 each) ampule sealing using HPLC–UV/vis analysis. The results showed that the flame had no effect on the concentration of DMTS during fire sealing (Figure 1).

### HPLC–UV/Vis Method Sensitivity

Different wavelengths were tested to determine the optimal absorbance for DMTS using HPLC–UV/vis analysis. The wavelength of 215 nm was chosen after eliminating the solvent cut-off of acetonitrile at 200 nm. This wavelength selection was consistent with prior HPLC–UV investigations of DMTS. The HPLC chromatogram of the F3-formulated DMTS with dimethyl disulfide (DMDS) internal standard (IS) is shown in Figure 2. DMTS and DMDS were eluted at 8.1 and 6.0 min, respectively. Calibration curves were constructed with calibration standards at 0.00, 0.01, 0.02, 0.03, and 0.04 mg/mL of DMTS and demonstrated to have a good linearity with an average R² value of 0.9991 (Figure 3). The limit of detection (LOD) and limit of quantification (LOQ) of the model were statistically determined to be 2.05 and 6.83 ng/mL, respectively. The quantification method of DMTS using HPLC–UV/vis demonstrated good intra- and inter-day precision and accuracy.
The highest intra- and inter-day precision values were 2.35 and 1.59% coefficient of variation (CV), and those for accuracy were 2.37 and 1.18%, respectively, which are within the acceptable range of ±15%, according to the FDA guidelines.

**Long-Term Stability of DMTS.** One of the objectives of this study was to determine the long-term storage stability of the F3-formulated DMTS stored in hermetically fire-sealed glass ampules. The DMTS ampules were kept in the refrigerator (4 °C) in a cupboard within a temperature-controlled room (22 °C) and in the oven (37 °C). Triplicates were sampled from each group at different time intervals over the period of a year. The concentrations of DMTS at each sampling point were determined using HPLC−UV/vis analysis, and the % (M/M) DMTS values were used to evaluate its long-term storage stability. The results showed that there was no measurable loss of DMTS throughout the entire period of the study when the ampules were stored at 4 or 22 °C. However, samples stored at 37 °C showed significant decrease after 5 months (Figure 4). In addition to the decrease in the concentration, the ampules had notable color change after 5 months of storage at 37 °C. As shown in Figure 5, the solution stored at 4 or 22 °C remained a clear light yellow color but the one stored at 37 °C displayed a dark brown discoloration, and the discoloration intensified toward the end of the study.

The HPLC chromatograms of all samples were carefully examined to determine whether chemical change occurred among the different storage temperatures throughout the entire storage period. The chromatograms of samples stored at 37 °C were first examined (Figure 6). The difference in peak heights and areas of DMTS and DMDS was evaluated, and the chromatograms at each time interval were compared to determine whether there were unknown peaks present. Observations of the chromatograms showed the gradual development of a new peak at 10.9 min from month 6 and another new peak at 15.1 min from month 8. These peaks were labeled as unknown 1 and unknown 2, respectively, until further analysis. To eliminate the possible sources of the unknown peaks due to contamination, additional samples from month 9 were analyzed using HPLC−UV/vis without the addition of IS (DMDS). In addition to the unknown peak 1 and 2, another new peak (unknown peak 3) was found at 6.0 min, eluting at the same retention time as DMDS. The chromatogram comparison of DMTS samples with and without the addition of IS showed that there were no significant differences in the peak areas and heights at the corresponding retention times.

### Table 1. Intra- and Inter-day Precision and Accuracy Values of the F3-formulated DMTS Quantification

| nominal concentration (µg/mL) | measured concentration (µg/mL) | standard deviation of the measured concentration (µg/mL) | precision (% CV) | accuracy (%) |
|-------------------------------|-------------------------------|----------------------------------------------------------|-----------------|-------------|
| Intra-Day (n = 5)             |                               |                                                          |                 |             |
| 10.0                          | 9.905                         | 0.019                                                    | 0.19            | 0.95        |
| 20.0                          | 19.68                         | 0.213                                                    | 1.08            | 1.60        |
| 30.0                          | 29.28                         | 0.688                                                    | 2.35            | 2.37        |
| 40.0                          | 40.04                         | 0.314                                                    | 0.78            | −0.11       |
| 50.0                          | 50.54                         | 0.622                                                    | 1.23            | −1.08       |
| Inter-Day (n = 5 at Day 1 & 2)|                               |                                                          |                 |             |
| 10.0                          | 10.12                         | 0.093                                                    | 0.92            | 1.18        |
| 20.0                          | 20.08                         | 0.309                                                    | 1.54            | 0.40        |
| 30.0                          | 30.10                         | 0.429                                                    | 1.42            | 0.35        |
| 40.0                          | 40.01                         | 0.637                                                    | 1.59            | −0.01       |
| 50.0                          | 49.88                         | 0.150                                                    | 0.30            | 0.24        |

Figure 4. One-year stability of the DMTS in the F3-formulation. Data are presented as mean ± sd (n = 3). Some error bars are not visible due to the low sd values.

Figure 5. Discoloration of the F3-formulated DMTS stored at 37 °C (left) while samples stored at 4 °C (right) and 22 °C (middle) remained unchanged after 12 months.

Figure 6. Comparison of HPLC chromatograms of F3-formulated DMTS samples at 37 °C in the month 9 sample with (bottom) and without (top) the addition of IS. Samples were analyzed using the mobile phase of acetonitrile/water = 65:35.
without IS is shown in Figure 6. DMTS analyses after month 9 (carried out without IS) showed significant decreases in the DMTS peak height and area, accompanied by growth of unknown peaks 1, 2, and 3. This suggested that the unknown peak 3 was not due to IS carryover from previous analysis, and it was likely that DMDS was a disproportionation/degradation product from DMTS during storage.

As previously discussed, no discoloration was observed for samples stored at 4 and 22 °C throughout the entire period of the study. Chromatograms of samples stored at 4 or 22 °C were examined to determine whether any chemical change occurred despite the lack of physical change. No unknown peaks were observed at 10.9 and 15.1 min in all samples analyzed with the addition of IS. Samples after 9 months of storage at both temperatures were also analyzed without the addition of IS, and no peaks were observed at 6.0 min (DMDS). These results suggested that the discoloration of the F3-formulated DMTS solution might be a good first indicator for the degradation of the antidote. Based on the absence of discoloration and chemical changes, this study was the first to confirm the long-term stability of the F3-formulated DMTS under refrigerated and room-temperature (22 °C) conditions.

Identification of Unknown Peaks from the Degraded DMTS Samples. Observations of unknown peaks led to the investigation of the identities of the possible degradation products. Due to the limitations of HPLC−UV/vis analysis without mass spectrometry, a GC−MS method was adopted in this part of the study to identify the unknown compounds. SPME was chosen to be the sample preparation method in lieu of regular injections. This technique provides a good filtering mechanism which ensures that only volatile components are injected to GC−MS. The polymer surfactants used for the F3-formulation are nonvolatile and present a potential risk for clogging the analytical column. A high split ratio of 600:1 was applied due to the high concentration of these samples and to prevent oversaturation. A sample from 22 °C was analyzed without the addition of IS to obtain a reference chromatogram for DMTS and to determine the potential thermal degradation caused by the high heat at the GC injection port. As shown in Figure 7, only one single peak of DMTS without any components were identified over 90% match upon the MS library search, and they were DMDS (2.20 min), DMTS (3.05 min), and dimethyl tetrasulfide (DM4S, 3.90 min) (Figure 8A). When compared to previous HPLC chromatograms in which two unknown peaks and a possible DMDS peak were noticed in the degraded samples, only one peak (DM4S) was noted in addition to the DMDS and the DMTS peaks in the GC−MS results. It is suspected that the disappearance of the last unknown peak might be due to the high split ratio (600:1) and highly diluted injection. As a result, a slightly modified GC−MS method was applied to analyze another degraded sample at a split ratio of 100:1. A peak identified as dimethyl pentasulfide (DMSS) was eluted after DM4S in addition to the DMTS and DMDS peaks (Figure 8B). The flow rate of the GC−MS method was also adjusted due to the change in the split ratio; therefore, a shift of the retention time of the compounds was observed. The result was not unexpected since DMTS is a member of the dialkyl polysulfides, which is thermally unstable and can degrade through disproportionation. Due to the lack of commercially available standard for DM4S and DMSS, direct identification of these two compounds was not possible. However, based on GC−MS analysis, it is confident to deduce that the unknown peaks 1 and 2 were DM4S and DMSS, respectively. In addition, the DMDS, DM4S, and DMSS observed were believed to be disproportionation products of DMTS that naturally formed at elevated temperatures.

Controlled Oxidation Study of DMTS. During the sealing process of the glass ampules, a small volume of air

![Figure 7](image_url)
was inevitably contained in the ampules, which could lead to the oxidation of the solutions of F3-formulated DMTS. It is important to determine whether the degradation products discovered in previous analyses were due to oxidation processes. As a result, an oxidation study of the solutions of F3-formulated DMTS was performed under controlled conditions using two strong oxidizing agents: meta-chloroperbenzoic acid (mCPBA) and H2O2 separately. For the reaction of DMTS and mCPBA, solutions were prepared in dichloromethane. For the reaction of DMTS and H2O2, DMTS was dissolved in 15% aqueous polysorbate 80 (poly 80) and mixed with 3% aqueous H2O2 solution. The prepared DMTS solutions were allowed to react with the oxidizing agents for 2 h at a low temperature of $-5^\circ$C. The DMTS solutions were analyzed right before mixing with the oxidizing agents and were sampled once a day afterward for 7 days. The oxidation products of mCPBA were analyzed both using HPLC−UV/vis and GC−MS while those of H2O2 were analyzed only with HPLC−UV/vis due to the high water content.

DMDS was not used as IS in the oxidation study due to its possible identity as one of the degradation products of DMTS. By the end of the 7-day study, four components were identified in the oxidation of DMTS with mCPBA using GC−MS analysis. As illustrated in Figure 9, the identified components were found to be DMTS (3.05 min), S-methyl methanethiosulfonate (SMMTS, 3.37 min), DM4S (3.90 min), and meta-chlorobenzoic acid (mCBA, 4.25 min). Based on previous oxidation studies using mCPBA, the presence of mCBA was a byproduct from the mCPBA solution.16 Multiple extractions with carbonate buffer solution (pH = 9.9) were performed after the oxidation reaction to remove the byproducts; however, the results showed that some amount of mCBA was still left in the reaction mixture. When the GC chromatograms of the oxidized samples were compared to those of the degraded samples stored at 37 °C, DMDS and DMSS were absent as the oxidation products. This phenomenon was further investigated by HPLC−UV/vis analysis.

Using HPLC−UV/vis analysis, six peaks were observed on the chromatograms at day 7 using mCPBA (Figure 10B). Based on the GC−MS results, commercially available SMMTS standard was analyzed using the HPLC−UV/vis method to aid with the identifications according to the retention time. Four peaks were identified as mCBA (2.0 min), SMMTS (3.7 min), dichloromethane (5.0 min), and DMTS (10.0 min). Despite the retention time shift, it is reasonable to conclude that the peaks observed at 13.7 min and 20.0 min were DM4S and DM5S, respectively, based on previous HPLC behaviors in the degraded samples stored at 37 °C. Figure 10A,B shows the HPLC chromatograms of the DMTS oxidation study using mCPBA at day = 0 and day = 7, respectively. (C) HPLC chromatograms of the DMTS oxidation study with H2O2 at day = 7. DMTS, DM4S, and DMSS were eluted at 10.0, 13.7, and 20.0 min, respectively. Samples were analyzed using the mobile phase of acetonitrile/water = 60:40.

Figure 9. GC−MS chromatogram of DMTS oxidation sample using mCPBA at day 7. A split ratio of 600:1 was applied in the oxidation study.

Figure 10. HPLC chromatograms of the DMTS oxidation study with mCPBA at (A) day = 0 and at (B) day = 7. At day = 7, six compounds, which included mCBA, SMMTS, dichloromethane (CH2Cl2), DMTS, DM4S, and DM5S were eluted at 2.2, 3.7, 5.0, 10.0, 13.7, and 20.0 min, respectively. (C) HPLC chromatograms of the DMTS oxidation study with H2O2 at day = 7. DMTS, DM4S, and DMSS were eluted at 10.0, 13.7, and 20.0 min. Samples were analyzed using the mobile phase of acetonitrile/water = 60:40.
DMDS was not detected using HPLC–UV/vis. In the analysis of the degraded samples stored at 37 °C, DMDS was suspected in the earlier discussion to be one of the degradation products of DMTS due to disproportionation reactions. Although DMDS is not expected to be produced via oxidation of DMTS by either mCPBA or H₂O₂ (Figure 10C), some DMDS might have been expected from simultaneous disproportionation reactions. However, no DMDS was observed in the intentionally induced oxidation study. It is hypothesized that a small amount of DMDS produced by disproportionation of DMTS was subsequently oxidized to DMTS, DM4S, and DM5S in the H₂O₂ experiment and to DMTS, DM4S, and DM5S and SMMTS in the mCPBA experiment (Figures 11 and 12). The peak heights and areas of SMMTS were also found to decrease with time through the oxidation study. This is likely due to the precipitations observed during the study period.

**CONCLUSIONS**

Recent investigational efforts focus on developing an intramuscular self-injector kit for ease of use in mass case scenarios. Previously, our laboratory developed, tested, and published lipid- and poly 80-based formulations. Ongoing efforts are focusing on newer, more advanced formulations. In order to produce a valid self-injector kit with a long storage life, the storage stability of the F3-formulated DMTS was investigated. The F3-formulated DMTS showed good long-term storage stability when it was kept at room temperature (22 °C) and under refrigeration. The samples stored at 4 and 22 °C displayed 100% (M/M) stability over a 12-month study. Samples stored at 37 °C showed 100% (M/M) stability only over the first 5-month period and gradually decreased to 30% (M/M) of the initial concentration by the end of the study. Therefore, it can be concluded that the F3-formulation coupled with the hermetic fire-sealing method can provide efficient long-term stability for DMTS when the formulation is kept either under refrigeration or at room-temperature (22 °C) conditions. Three major degradation products were identified in this study: DMDS, DM4S, and DM5S. All three of the compounds were proposed to be the disproportionation products of DMTS. DMDS was found preferably under natural degradation while DM4S and DM5S were preferable products when strong oxidizing agents were present. DMDS was not recommended in future DMTS studies as an IS due to its involvement in the degradation process. In January 2020, Buchstav and Kamysny reported parallel findings of the disproportionation of DMTS to form DMDS and DM4S. HPLC–UV/vis and GC–MS/SPME analyses revealed good agreement between the oxidized subset of disproportionation products of the stability study and the direct oxidation reactions.

**METHODS AND MATERIALS**

**Chemicals and Reagents.** All chemicals used were of at least HPLC grade. DMTS, DMDS, calcium carbonate, calcium bicarbonate, hexane, ethyl acetate (EtOAc), hydrogen peroxide (H₂O₂), acetone, ethanol, and SMMTS were purchased from Sigma-Aldrich (St. Louis, MO, USA). mCPBA, acetonitrile, and water were purchased from Acros Organics (Thermo Fisher Scientific, Waltham, MA, USA). Poly 80 was obtained from Alfa Aesar (Tewksbury, MA, USA), and sorbitan oleate 80 (Span 80) was purchased from TCI America (Portland, OR, USA). Except where other temperatures are noted, all experiments were carried out in a temperature-controlled laboratory at 22 °C.

**Preparation of the F3-Formulated DMTS.** The F3-formulation of DMTS was prepared using the protocol provided by the Southwest Research Institute (San Antonio, TX). It contains two surfactants, poly 80 and Span 80 that serve as the solvent. The formulation contains no additional solvent. The poly 80 (99.57 g) and Span 80 (33.20 g) were placed into a glass bottle and vortexed for 5 min, following the addition of DMTS (88.5 g) to prepare the formulation stock solution (100 mL, 400 mg/mL). The resulting mixture was vortexed for 5 min and then was subjected to auto-vortex at 2000 rpm (Heidolph North America, Wood Dale, IL) for 30 min. The end product of the stock F3-formulation of DMTS was a clear dark yellow solution.

**Stability Studies.** The prepared stock solutions of F3-formulated DMTS were aliquoted in glass ampules that were hermetically sealed with an ampule sealer under a high-temperature flame. Following sealing, the samples were divided into three groups (63 in each group, total of 189 samples) and were stored at different temperatures: 4 °C (kept in the refrigerator), 22 °C (kept in the storage cupboard), and 37 °C (kept in the oven), as illustrated in Figure 13. Three ampules from each group were sampled daily in the first week, weekly for the remainder of the first month, and monthly thereafter for a period of 1 year. At each sampling point, the remaining DMTS content was determined. The DMTS concentration of the freshly prepared F3-formulated DMTS was analyzed and marked as day 0 of the long-term stability study. Samples stored at 37 °C after 6 months were subjected to additional GC–MS/SPME analysis to identify the degradation products. The samples were directly transferred from the ampules to the GC–MS vials for analysis.

For the quantification of the DMTS at different storage conditions, the stock F3-formulated DMTS (400 mg/mL) was diluted with ethanol to obtain a working solution of 0.05 mg/
HPLC–UV/Vis Analysis. HPLC–UV/vis analysis was performed using the Dionex UltiMate 3000 UHPLC system coupled with a diode array detector for UV/vis detection (Thermo Scientific, Waltham, MA). The instrument was equipped with a Luna C8 column (250 × 4.60 mm, pore size of 100 Å, particle size of 5 μm) (Phenomenex, Torrance, CA). The HPLC–UV/vis method was adopted from the study by Rockwood et al. with modifications to adapt to the low concentrations of DMTS in aqueous medium. For quantification of DMTS, the mobile phase consisted of 35% (v/v) water and 65% (v/v) acetonitrile with a 1 mL/min flow rate in an isocratic elution mode. The absorbance of the analytes was monitored at 215 nm. The injection volume was set to 40 μL, and the total run time was 30 min. In the oxidation study, the prepared oxidation products were analyzed with HPLC–UV/vis at the same flow rate, but the mobile phase content was modified to 60% (v/v) acetonitrile and 40% (v/v) water in the isocratic elution mode. The injection volume for oxidation analysis was set to 10 μL, and the total run time was 25 min. Chromatographic analysis was performed with the Chromeleon chromatographic data system (version 7.2, Thermo Scientific, Waltham, MA).

GC–MS/SPME Analysis. Stability samples stored at 37 °C after 6 months, and samples of the oxidation reaction using mCPBA were analyzed using GC–MS/SPME. Analyses were conducted using the Agilent 7980A GC coupled with a 5975C MS detector and a 7693 autosampler (Agilent, Santa Clara, CA). The column used was a DB-5MS column (30 m × 0.25 mm, 1 cm in length, 25 μm) with helium as the carrier gas. The SPME fiber used was a polydimethylsiloxane fiber (100 μm, 1 cm in length, 24 Ga purchased from Agilent Santa Clara, CA) with the 24 Ga fiber assembly (Supelco, Bellefonte, PA). The fiber was exposed to the sample for 30 s at room temperature (22 °C) and was then desorbed in the injector at a temperature of 180 °C. The split ratio was 600:1. The GC oven temperature was programmed to hold at 50 °C for 1 min, then was elevated at a rate of 60 °C/min up to 280 °C and held for 3 min. The MS was operated in the scan mode. The data obtained from GC–MS/SPME analysis were analyzed using the ChemStation software (Agilent, Santa Clara, CA).

Oxidation Studies. The protocol for the oxidation study was adopted from the study by Auger et al. with modifications. For oxidation of DMTS with mCPBA or H2O2, the study was conducted using the same procedures. Namely, DMTS (1.26 g, 10 mmol) was first mixed with 5 mL of dichloromethane (or 15% poly 80 in the case of H2O2) and stirred for 5 min. Then, a solution of either mCPBA (1.89 g, 11 mmol) dissolved in 20 mL of dichloromethane or H2O2 (0.37 g, 11 mmol) dissolved in water was slowly added to the DMTS solution and was allowed to react for 2 h at a low temperature (−5 to 0 °C). The DMTS solution was analyzed before mixing with the oxidizing agent at day = 0. After the 2 h reaction, the mixture was stored at room temperature (22 °C) and sampled for analysis at day = 1−7.

The oxidation mixture (500 μL), for both mCPBA and H2O2, was transferred to an Eppendorf tube and was subjected to liquid–liquid extraction three times with 500 μL of 0.1 M sodium bicarbonate buffer solution (pH 10.0). The organic phases were pooled, and 50 μL was transferred to a separate tube, diluted with 450 μL of ethanol, and auto-vortexed for 5 min. The vortexed sample (70 μL) was further diluted with 630 μL of ethanol for HPLC–UV/vis analysis. In addition to
HPLC–UV/vis analysis, GC–MS/SPME analysis was also performed on the reaction mixture involving mCPBA. The final diluted samples were first dried with anhydrous sodium sulfate and directly subjected to GC–MS/SPME analysis. Due to their high water content, the H₂O₂ reaction mixture samples were only analyzed using HPLC–UV/vis.

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**Notes**

The authors declare the following competing financial interest(s): Two patents by two coauthors, related to DMTS are: (1) Gary A. Rockwood, Ilona Petrikovics (SHSU) and Steven I. Baskin (USAMRICD): Dimethyl Trisulfide as a Cyanide Antidote. U.S. Patent No. 9,375,407, 2016, (2) Ilona Petrikovics and Kristof Kovacs (SHSU). Formulations of Dimethyl Trisulfide for Use as a Cyanide Antidote. U.S. Patent No. 9,456,996, 2016.

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