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Optimization and Structural Characterization of Dimethyl Trisulfide (DMTS) Oxidation Product

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ABSTRACT

Cyanide poisoning is a public concern, and there are many shortfalls in current cyanide treatments. Dimethyl trisulfide (DMTS) is a cyanide antidote candidate that overcomes these shortfalls. Currently, there are limited published reports related to the analysis of DMTS. Therefore, an analytical method to detect and analyze DMTS from a biological matrix is vital for it to become available as a therapeutic agent against cyanide poisoning. The motivation of this project is to develop an HPLC-MS/MS method for analysis of DMTS and its degradation products; however, DMTS is difficult to ionize, a requirement for MS analysis, due to its nonpolar nature. In this study, DMTS was oxidized to a more polar compound that should enable its MS-MS analysis. The oxidation reaction was optimized to maximize product yield and, therefore, improve the accuracy of the analytical technique. The optimized oxidation reaction increased the yield of oxidized DMTS by 17.4% and decreased the amount of un-oxidized DMTS by 88.5%. In addition, initial characterization of the reaction product was preformed, using GC-MS. The preliminary results indicated the DMTS was fully oxidized.

INTRODUCTION

Cyanide is a potent metabolic poison. Upon exposure, cyanide enters mitochondria and binds to cytochrome c stopping production of ATP, effectively killing the cell[1]. Exposure to

cyanide can occur due to consumption of food containing cyanide, such as cassava, almonds, and apricot seeds. Other sources of exposure include anthropogenic sources: mining industry, smoking cigarettes, and smoke inhalation from fires. In addition, exposure can result from malicious use of cyanide, such as a terrorist weapon. Due to the numerous sources of exposure, there is a need for an efficient cyanide antidote.

Several types of cyanide antidotes have been developed. One class of cyanide antidotes is sulfur donors. According to the Centers for Disease Control and Prevention (CDC) guidelines from October 2014, sodium thiosulfate is the primary sulfur donor based antidote for cyanide poisoning[2]. Sodium thiosulfate is utilized by the enzyme rhodanese to donate sulfhydryl groups that bind with cyanide to form the less toxic thiocyanate, preventing the binding to cytochrome c[1].

Dimethyl trisulfide (DMTS) is a potential novel cyanide antidote that acts as a sulfur donor. DMTS does not require rhodansese to form thiocyanate in the body. DMTS is an ideal candidate for intramuscular injection, creating an antidote that could be readily administered in a mass exposure scenario[3].

In order for DMTS to be available as an FDA approved cyanide antidote, a method must be developed to analyze DMTS and DMTS degradation products in blood. A High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS) method is highly desirable for measurement of both DMTS and DMTS degradation products. Due to the non-polar nature of DMTS, it does not ionize in electrospray ionization and cannot be detected in Electrospray Ionization Mass Spectrometry (ESI-MS). Oxidizing the DMTS molecule to a polar molecule could allow for detection in an ESI-MS instrument.

METHODS

Optimization of Oxidation Reaction

DMTS solution (19.8 mM) was prepared by diluting a 396 mM stock solution with HPLC grade methanol (MeOH). The initial oxidation method was as follows: 1350 µL of deionized H_2O, 200 µL of DMTS sample, 400 µL of 30% H_2O_2, and 50 µL of 1 mM nitric acid were added, along with a stir bar, to the reaction vial. The reaction vials were placed in

a heat block with a thermometer on a hot plate. The power of the hot plate was adjusted until the temperature of the thermometer on the heat block stabilized at 30 °C. The reaction proceeded for 1 hour.

Oxidation parameters, such as temperature, reaction time, and amount of nitric acid were systematically changed to maximize the product (oxidized DMTS) yield. Triplicates of samples were used for analysis.

After oxidation, the oxidized DMTS was extracted by adding 600 µL of ethyl acetate to reaction vials, mixing, and then settling. The organic layer was separated and removed from each vial. The organic layer was washed twice with 500 µL of H_2O, then separated and filtered through a Teflon filter and analyzed using an Agilent Technologies 1200 Series HPLC-UV-Vis instrument. Gradient elution was used with 30% MeOH and 70% H_2O for 4 minutes, linearly increased to 100% MeOH over 5 minutes, held constant for 2 minutes, and linearly decreased to 30% MeOH over 3 minutes at a flow rate of 0.35 mL/minute. A multiwavelength UV detector at wavelength 280 nm was used to measure the absorbance. The column was a 2.1 x 150 mm ZORBAX Eclipse XDB-C-18 reverse phase column, with a 160 Å pore size.

Isolation of Oxidation Product by Column Chromatography

Before structural characterization of oxidized DMTS, a normal phase gravity column (7 cm in length, 1.5 cm diameter) containing 60Å silica beads (230-400 mesh particle size) was used to separate the oxidized DMTS from the other components of the reaction vial. The mobile phase was ethyl acetate.

Gas chromatography-mass spectrometry (GC-MS)

An Agilent Technologies 5975B Inert XL EI/CI GC/MSD system with an Agilent Technologies 7683B Series injector and a gas chromatography column with a length of 30 m and internal diameter of 0.250 mm was used to analyze the isolated oxidation product. The GC-MS instrument had an inlet temperature of 150 °C and gas flow rate of 58 mL/min. The oven temperature was controlled starting from 50 °C for 1 minute with a ramp of 120 °C/min for 2 minutes, and held at 290 °C for 1 minute.

Data Analysis

The percent change in peak area for the oxidation product and DMTS was calculated using Equation 1. The standard deviation of the percent change was calculated using Equations 2 and 3.

$$\%\ change\ in\ Peak\ area = \frac{Average\ Peak\ area_{final} - Average\ peak\ area_{initial}}{Average\ peak\ area_{initial}} \times 100\% \quad (1)$$

$$Std.dev\ of\ the\ percent\ change\ (s_f) = \sqrt{s_{initial}^2 + s_{final}^2} \quad (2)$$

$$Percent\ Std.dev\ of\ the\ percent\ change\ \left(s_f\right) = \frac{s_f}{Average\ peak\ area_{initial}} \times 100\% \quad (3)$$

Equations 1-3: The equations above show calculations performed during data analysis.

RESULTS

Through systematic variation of the initial oxidation reaction parameters, the effect of each parameter on oxidized DMTS formation was determined. The effects of changing parameters can be seen in Table 1. The only parameter that significantly increased the oxidized DMTS yield was an increase in the reaction time. When the temperature was increased from 30 °C to 45 °C, the peak area of oxidized DMTS decreased slightly (by 6%). When the temperature was increased to 60 °C, the peak area of oxidized DMTS decreased by 67%.

Table 1: The percent change of peak area and standard deviation of DMTS and oxidized DMTS with changing reaction parameters compared to initial reaction conditions.

Changed Parameter	**% Change in Oxidized DMTS**	**% Change in DMTS**
Time: 1 hr to 3 hr	17.4 ± 7.1%	-88.5 ± 3.2%
Temp: 30 °C to 45 °C	-6.6 ± 4.5%	-77.9 ± 3.7%
Temp: 30 °C to 60 °C	-66.8 ± 29.1%	-97.9 ± 4.0%
Nitric Acid Volume: 50 µL to 25 µL	-39.3 ± 5.7%	60.7 ± 24.3%
Nitric Acid Volume: 50 µL to 100 µL	-17.7 ± 28.8%	-39.0 ± 33.6%

The optimized oxidation conditions are as follows: 1350 µL of deionized H_2O, 200 µL of DMTS sample, 400 µL of 30% H_2O_2, and 50 µL of 1 mM nitric acid reacted for 3 hours at 30 °C. Optimized oxidation reaction conditions led to an increase of 17.4 ± 7.1% of oxidized DMTS. The optimized reaction conditions decreased DMTS by 88.5 ± 3.2 %. Figure 1 illustrates a comparison between optimized reaction conditions and initial reaction conditions.

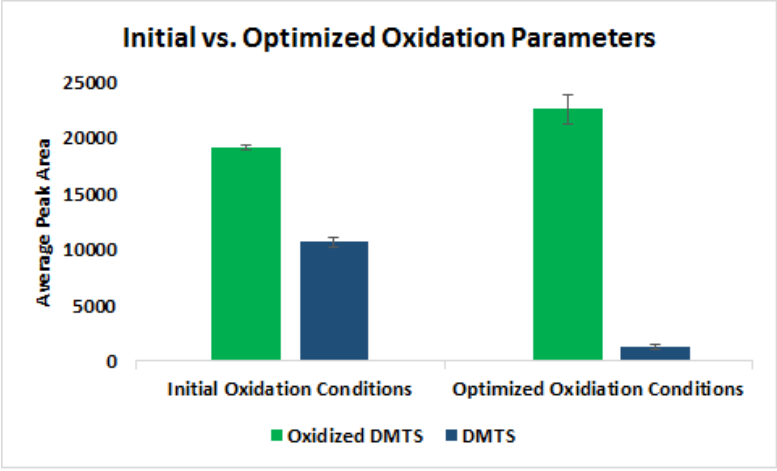


Figure 1: Initial vs. Optimized Oxidization Parameters effect on DMTS and Oxidized DMTS.

The mass spectrum of the oxidation product peak in GC-MS contained ions of mass/charge (m/z) 126, 147, 158, 175, 189, 207, and 221, see Figure 2.

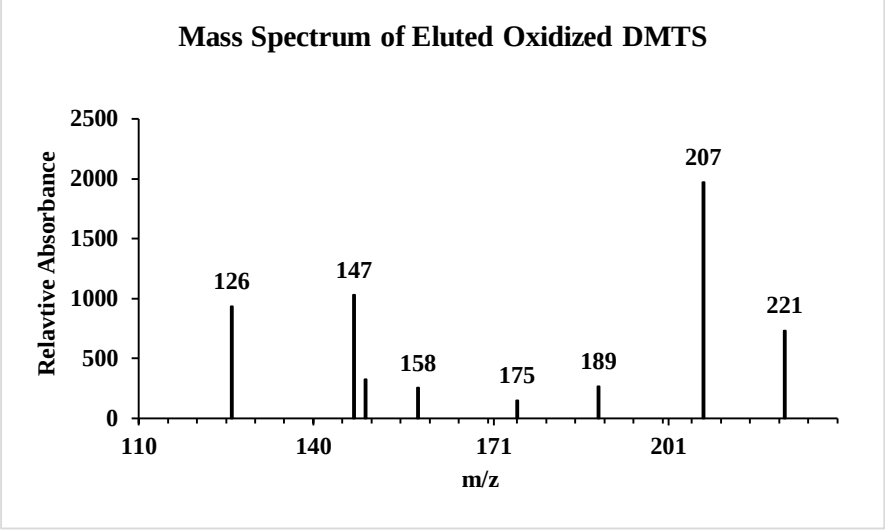


Figure 2: Mass Spectrum of the GC peak at 3.465 min.

DISCUSSION

The loss of product with increasing temperature is likely attributed to the volatility of DMTS and oxidized DMTS, as well as thermal degradation of these compounds. It was also noticed that increasing the reaction time increased the yield of oxidized DMTS; this is likely due to the fact that reaction equilibrium is not reached within 1 hour and extending the reaction to 3 hours allows the reaction to approach equilibrium.

The MS fragmentation pattern of oxidized DMTS suggests that the structure in Figure 3 is likely that structure. The m/z ions of 147, 158, 175, 189, 207, and 221 likely correspond to fragments after the original molecule lost methyl groups, hydrogen atoms, sulfur atoms, and oxygen atoms. For example, the m/z ion of 207 corresponds to a fragment with the loss of a methyl group, and the ion at 175 likely corresponds to a fragment resulting from the loss of a methyl group and two oxygen atoms. The probable structures for the fragment ions can be seen in Table 2. The proposed structure for oxidized DMTS can explain the data obtained through mass spectrometry. However, the proposed molecule of the oxidized DMTS cannot explain all of the data in the mass spectrum in Figure 2. For example, the 147 m/z ion does not fit the proposed structure. Further study of the oxidation product is needed to confirm the proposed structure.

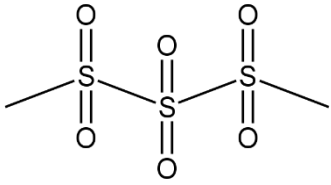
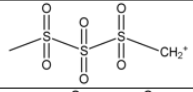
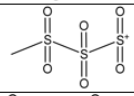
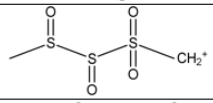
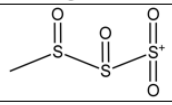
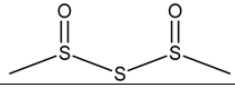


Figure 3: Predicted oxidized DMTS structure,

Table 2: Fragment mass/charge (m/z) ratio from the mass spectrum and predicted structural assignments.

m/z	Predicted Structure
221	
207	
189	
175	
158	

CONCLUSIONS

Increasing the time of the oxidation reaction allowed for an increase of 17.4% for oxidized DMTS. A preliminary structure of oxidized DMTS was determined. Future work will be needed to test these optimized oxidization parameters on lower concentrations of DMTS. Further characterization of oxidized DMTS will also be conducted using LC-MS/MS and ultimately an LC-MS/MS method will be developed. The complete development of this method provides a necessary tool in the approval of DMTS as a cyanide antidote.

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