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Determination of Mustard Gas Hydrolysis Products Thiodiglycol and Thiodiglycol Sulfoxide by Gas Chromatography-Tandem Mass Spectrometry after Trifluoroacetylation

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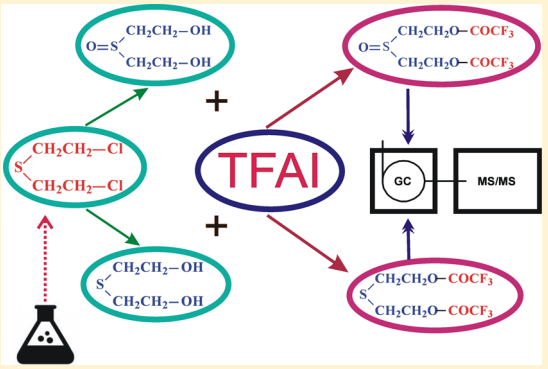
ABSTRACT: A method for detecting mustard gas degradation products thiodiglycol (TDG) and thiodiglycol sulfoxide (TDGO) in water and sediment samples using gas chromatography-tandem mass spectrometry (GC-MS/MS) after derivatization with 1-(trifluoroacetyl)imidazole (TFAI) was described. Selected reaction monitoring mode (SRM) of tandem mass spectrometry was developed for analysis of TDG and TDGO derivatives while analysis by gas chromatography-atomic emission detector (GC-AED) was performed using the 181 nm sulfur canal. TFAI derivatization conditions were optimized and the method validated. Two derivatization agents were compared, TFAI and *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA), where derivatization using TFAI occurred quicker and under milder conditions than using BSTFA. Water samples containing TDG and TDGO were evaporated to dryness under nitrogen, dissolved in organic solvent followed by reaction with TFAI. The limit of detection (LOD) for TDG and TDGO were 0.01 and 5 ng/mL, respectively. The limit of detection for TDG was decreased by two orders of magnitude if TFAI is used for derivatization rather than silyl derivatizing agents (e.g., BSTFA). TFAI has three major advantages in comparison to BSTFA, the first is much higher sensitvity, the second is a very clean background of chromatograms, and the last one is very mild conditions of derivatization. Moreover, by-products are not formed during derivatization of TDGO by TFAI in comparison to derivatization by silylating agents.



The Chemical Weapons Convention (CWC) was enacted in 1997 which prohibits the development, production, stockpiling, and use of chemical weapons as well as addressing their destruction.[1] Development of analytical methods suitable for analysis of chemical warfare agents (CWAs) and their degradation products has gained importance in verification of CWC compliance,[2] where verification procedures involve collection and analysis of samples from routine, alleged use, or challenge inspections.[3]

Bis(2-chloroethyl)sulfide (sulfur mustard, mustard gas or yperite) is a blistering agent widely used during WWI, produced during WWII, and also used during the Iran-Iraq conflict in the 1980s.[4] As sulfur mustard is easily hydrolyzed and/or oxidized in water and moist soils, the analysis of degradation products (see Figure 1) is accomplished by liquid chromatography[5] and gas chromatography (GC) coupled with various detectors including pulsed flame photometric,[6] mass spectrometer,[7] atomic emission,[8] and tandem mass spectrometer.[9]

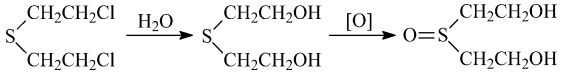


Figure 1. Scheme of the sulfur mustard degradation reaction in the environment.

In environmental and biological matrixes, sulfur mustard is easily hydrolyzed to thiodiglycol (TDG), a relatively stable, low volatility, and low toxicity compound,[10] while TDG remaining in environmental or biological samples may be further oxidized to thiodiglycol sulfoxide (TDGO).[11,12] TDGO is also a metabolic transformation product of yperite and has been found in the urine of experimental animals and of persons accidentally exposed to sulfur mustard.[13] For the analysis of TDG and TDGO in biomedical samples, limits of detection in the range of 1 ng/mL are often required.[14] Therefore, if the use of sulfur mustard is to be proven, sensitive and precise TDG and TDGO determination methods for environmental and biomedical samples are required to verify the presence of sulfur mustard.

It is estimated that after WWII, tens of thousands of tons of various chemical munitions from German stockpiles were sunk in the Baltic Sea under agreement with Allied countries, of which approximately two-thirds of that material was sulfur

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mustard. Seventy years after the end of the war, chemical munitions are still leaking CWAs into the marine environment and the CHEMSEA project is devoted to risk assessment of these chemical munitions dumped into the Baltic Sea.[15,16] One of the goals is to conduct chemical analyses for sulfur mustard and its degradation products (such as TDG and TDGO) in sediments from dumpsites (Gotland deep, Gdansk deep, and Bornholm deep), which requires sensitive detection methods for very low TDG and TDGO levels in water and bottom sediments.

TDG and TDG could be most conveniently analyzed by LC−MS although limits of detection are unsatisfying (10 ng/mL in clean water)[17] and this method is not suitable for trace analysis in the case of biomedical samples. Underivatized TDG and TDGO can be analyzed by GC, but peak shapes are not ideal and derivatization is required for analysis of concentrations below 1 ppm.[14] Modification of TDG and TDGO into more volatile and less polar derivatives allows quantitative and qualitative analysis by GC. Silylating agents are most commonly used for derivatization of TDG and TDGO,[18] and the most common derivatizing agents used for analysis of sulfur mustard degradation products are *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) (see Figure 2) and *N*-(*tert*-butyldimethylsilyl)-

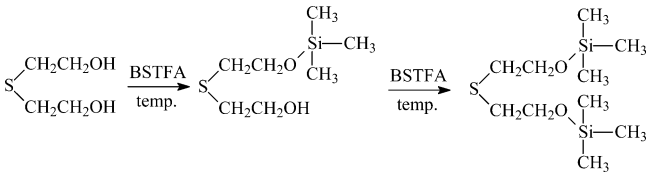

Figure 2. Derivatization reaction of thiodiglycol (TDG) with *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA).

N-methyltrifluoroacetamide (MTBSTFA).[18] These derivatization reactions are used by the majority of laboratories analyzing samples for the presence of CWAs and their degradation products and by laboratories participating in proficiency testing for the Organization for the Prohibition of Chemical Weapons (OPCW).[19]

The universal character of silylation is advantageous because it allows analysis of a large group of compounds in the same sample, but is a major drawback when analyzing environmental samples such as soil, water, or sediments. For examples of OPCW analyses, you can look for anything from the CWC Schedule.[1] Derivatization of compounds from water or sediment samples leads to formation of artifacts in large quantities compared to that of the expected analytes, which leads to multiple peaks in gas chromatographic analysis.[20] Compounds possessing carboxyl and hydroxyl groups undergo silylation and yield chromatograms obscured by a strong background.

Another disadvantage of silylation reagents is their high sensitivity to the presence of even minor amounts of water in the sample. Derivatization using BSTFA or MTBSTFA requires dry, moisture-free conditions and use of aprotic organic solvents,[21] as the derivatizing agent and derivatization products are decomposed in the presence of water at various hydrolysis rates.[22] Reagents that introduce a *t*-butyldimethylsilyl (TBDMS) group in place of the TMS group were developed to impart greater hydrolytic stability to derivatives with the added advantage of yielding distinctive fragmentation patterns useful for a variety of GC-MS applications.

For the analysis of TDG in blood or urine, lower limits of detection are normally required and nonsilyl methods of derivatization should be used. Black and Read[23] developed a method that converted TDG to its bis-pentafluorobenzoyl derivative with a detection limit of 1 ng/mL, while thiodiglycol was analyzed as a bis(heptafluorobutyryl) derivative by Jakubowski et al.,[24] where TDG in urine could be detected down to concentrations of 1 ng/mL. This method has since been used to detect TDG in the urine of a casualty accidentally exposed by sulfur mustard.[25]

TDGO analysis is a complex issue using both LC as well as GC. TDGO presents two problems for analysis by GC. The first is isolation from the aqueous matrix, the second is derivatization. TDGO, because of the highly polarized nature of the S=O bond, is much more polar than TDG or TDG Sulphone, and extraction from an aqueous solution is difficult other than by evaporation to dryness. Black and Read derivatized TDGO with pentafluorobenzoyl chloride.[26] During the experiment, it was observed that the derivative formed was the same as that from TDG, i.e., the sulfoxide function was reduced. This made it difficult to differentiate TDGO from TDG at trace levels other than by selective extraction. Black et al.[27] have investigated the derivatizatotion of TDGO with a number of reagents. The derivatization of TDGO is much more complex than derivatization of TDG because of the presence of sulfoxide oxygen in the molecule which is an additional nucleophilic site for reaction. Three major types of derivatives are formed, depending on the reagent and conditions. These result from simple derivatization with preservation of the sulfoxide function, reduction to the corresponding TDG derivative, and Pummerer-type rearrangement[28] to derivatives of 1-hydroxy-TDG, which undergo elimination to form olefinic products. For example, the reaction of TDGO with heptafluorobutyric anhydride (HFBA) or trifluoroacetic anhydride (TFAA) rendered primarily derivatives formed by a Pummerer-type rearrangement and products of elimination. On the other hand heptafluorobutyrylimidazole (HFBI), which has the advantage of not releasing acid during the reaction, rendered the sulfoxide derivative as the predominant product. HFBI was evaluated[29] and found to give much reduced chemical background in trace analysis compared to pentafluorobenzoyl chloride. During the derivatization of the TDGO with heptafluorobutyryl chloride and trimethylsilyl cyanide it has been observed that TDGO was reduced to TDG and then the derivative of the TDG was created. It might be supposed that TFAI would be as good of a derivatizing agent as HFBI or better. One of the aims of the present study was also verification of TFAI derivatization potential.

Pardasani et al.[30] have shown that 1-(trifluoroacetyl)-imidazole (TFAI) may be an excellent derivatization reagent for the analysis of TDG and TDGO by gas chromatography with mass spectrometry (reaction shown at Figure 3). In the present study, TDG and TDGO derivatization using TFAI was further developed and optimized by studying the effects of

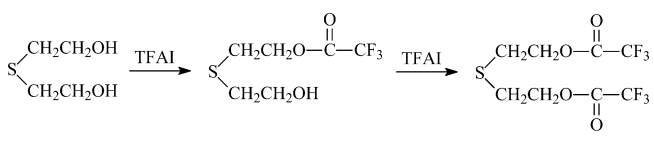

Figure 3. Derivatization reaction of thiodiglycol (TDG) with 1-(trifluoroacetyl)imidazole (TFAI).

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solvent type, derivatization time and temperature, as well as the influence of water on the reaction and derivatization product stability as a function of storage time. An analytical method was developed for evaluating TDG and TDGO using gas chromatography-tandem mass spectrometry (GC-MS/MS) with selected reaction monitoring (SRM).

EXPERIMENTAL SECTION

Equipment. Quantitative and qualitative analyses were performed using a HP 6890 gas chromatograph equipped with an atomic emission detector (AED, Hewlett-Packard HP G2350A) controlled by the Chemstation HP 35920A software, and a 7890A GC coupled with a tandem mass spectrometer Agilent Technologies 7000 GC/MS triple quadrupole (Agilent Technologies, Palo Alto, CA) controlled by MassHunter software.

Reagents. Methylene chloride, hexane, propylene carbonate, acetonitrile, dioxane, anhydrous magnesium sulfate(VI), and ethyl acetate were obtained from POCH (Gliwice, Poland) while 1-(trifluoroacetyl)imidazole, *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide, and thiodiglycol from Sigma-Aldrich (St. Louis, MO). Thiodiglycol oxide was synthesized[31] and purity verified by liquid chromatography−mass spectrometry.

Chromatographic Analysis. Parameters for GC coupled with AED: plasma chamber and transfer-line temperatures were 270 °C, injector temperature was 260 °C, split-ratio was five-to-one (5:1) for the calibration curve and fifty-to-one (50:1) in other cases, time for solvent purging from the detector was 2−7 min, helium was used as the carrier gas at a flow rate of 1 mL/min, and hydrogen, oxygen, and methane-nitrogen (10:90 v/v %) were used as reagent gases. A HP-5 capillary column (30 m, 0.25 mm and 0.25 μm) with the stationary phase containing 5% diphenyl- and 95% dimethylpolysiloxane was used for the analysis of silyl- and trifluoroacetyl TDG and TDGO derivatives, where the column was heated from 70 to 240 °C at the rate of 15 °C/min and the final temperature maintained for 5 min.

Operational parameters for the gas chromatograph-tandem mass spectrometer were injector temperature of 260 °C, transfer-line temperature of 250 °C, splitless for the calibration curve and 200:1 in other cases, a solvent delay time of 7 min, and helium as the carrier gas at 1 mL/min. Nitrogen was used as the collision gas at a flow of 1.5 mL/min, and helium quench gas was added to the collision chamber at 2.25 mL/min. The HP-5MS capillary column (30 m, 0.25 mm, 0.25 μm) with 5% diphenyl- and 95% dimethylpolysiloxane solid phase was used for analysis of TDG and TDGO derivatives, where the column was heated from 70 to 240 °C at the rate of 15 °C/min.

Thiodiglycol and Thiodiglycol Sulfoxide Derivatization Procedure. *Derivatization with TFAI in Organic Solvent.* Derivatization of TDG and TDGO with *N*-(trifluoroacetyl)imidazole (TFAI) was accomplished by adding 0.5 mL of TDG or TDGO 100 pbb solution in a selected organic solvent (methylene chloride, hexane, acetonitrile, ethyl acetate, dioxane, or propylene carbonate) and adding 0.5 mL of 10% TFAI solution in the same organic solvent. The mixture was shaken for 1 min at 2000 strokes per minute and heated at a 30 °C (preselected temperature) for 1 min.

Thiodiglycol and Thiodiglycol Sulfoxide Derivatization with TFAI in Water Samples. Aqueous TDG or TDGO solutions (2−5 mL) of a 100 ppb concentration were evaporated to dryness at 30 °C under a free flow of nitrogen. The dry residue was dissolved in 2 mL of a selected organic solvent (methylene chloride, hexane, acetonitrile, ethyl acetate, dioxane, or propylene carbonate) and 0.5 mL of a 10% TFAI solution in the same organic solvent was added, shaken for 1 min at 2000 strokes per minute at room temperature, and analyzed.

Derivatization with BSTFA in an Organic Solvent. A 100 ppb solution of TDG or TDGO (in a 2 mL vial) was dissolved in 1 mL of acetonitrile, the vial capped with a screw cap fitted with a Teflon-coated silicone septum, and 50 μL of BSTFA added by injection. The vial was heated at 70 °C for 60 min and the resulting solution analyzed by GC.

Derivatization with BSTFA in Water Samples. Acetonitrile (1 mL) was added to the dry residue concentrated from TDG or TDGO 100 ppb aqueous samples and purged with nitrogen to ensure dryness (2 mL reaction vial). The vial was capped using a screw cap fitted with a Teflon-coated silicone septum and 50 μL of BSTFA added by injection. The vial was sonicated for 5 min to ensure mixing, heated at 70 °C for 60 min, and the resulting solution analyzed by GC.

Optimization of the Derivatization Reaction Conditions. Reaction parameters for derivatization with 1-(trifluoroacetyl)-imidazole (TFAI) were optimized for a 1 mg/mL solution of thiodiglycol (TDG) in an the organic solvent, and these optimized reaction conditions are also used for thiodiglycol sulfoxide (TDGO).

Selection of Organic Solvent. The effect of organic solvents (methylene chloride, hexane, acetonitrile, ethyl acetate, dioxane, and propylene carbonate) on rate and efficiency of the TDG derivatization reaction with *N*-(trifluoroacetyl)imidazole were assessed at a temperature of 30 °C. For each solvent, the chromatographic analysis were made in the following time intervals: 1, 5, 10, 15, 20, 30, 60, and 90 min.

Selection of Time and Temperature. The effects of temperature (25−100 °C) and time (1, 5, 10, 15, 20, 30, 60, 90 min and every 2 h, the end point was 12 h) on the rate and efficiency of the TDG derivatization with TFAI were evaluated.

Water Influence. The effects of water on the rate and efficiency of TDG derivatization reactions with *N*-(trifluoroacetyl)imidazole were also studied. For that purpose, four vials containing 1 mg/mL TDG solution in acetonitrile at 0%, 1%, 5%, and 10% water were prepared. The reaction was carried out at 30 °C, 25 μL of TFAI was added to each mixtures, shaken for 1 min at 2000 strokes per minute, and analyzed by GC-MS/MS.

Direct Extraction from Water. Whether TDG derivatization was possible during extraction of aqueous samples with selected solvents containing the derivatizing agent was evaluated. Derivatization combined with extraction was carried out with 2 mL of aqueous samples, at a TDG concentration of 1 mg/mL. The extraction was done using 2 mL of solvent (methylene chloride, ethyl acetate, or hexane) with 50 μL of TFAI and shaking for 10 s, 1 min, 2 min, or 5 min.

Influence of Water on Stability of Derivative. As published data indicated, the derivatizing agents and derivatives were easily decomposed by water in the sample,[32] and the effect of water on the stability of derivatives formed in the TDG and TFAI reaction was assessed. Acetonitrile solution (1 mL) containing the TDG derivatization reaction product (1 mL vial) was sampled at 10 min intervals. After 50 min, 1 μL water was added and 70 min after, 2 μL water was added and the mixture monitored for 80 min.

Stability of Derivative. Samples containing TDG and TDGO derivatized with TFAI were evaluated for storage

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stability. Solutions at two concentration levels were prepared for both substances, where the high concentration solution was 1 mg/mL and the low concentration sample was 1 μg/mL. These solutions were stored at room temperature and analyzed for 4 days at appropriate intervals (from 2 to 10 h).

Spiking of Sediment Samples from the Baltic Sea. The sediment samples were spiked with TDG and TDGO water solution (1 ppm), and the amount of sediment was 10 g. Then the samples were homogenized and shaken for 2 h, after that the samples were placed in the refrigerator for 24 h. Then the samples were prepared for chromatographic analysis.

Preparation of Sediment Samples from the Baltic Sea. At the beginning of the sample preparation, the samples were taken out of the freezer and left to stand and melt under a hood with good ventilation for about 4 h. Then a 50 ± 1 g-portion of the sediment was weighed into a Falcon tube. The sediment was homogenized by mixing before taking an aliquot. The remaining sediment was stored in a freezer for possible later use. Next, the sample was centrifuged for 10 min at 42 000 rpm, excess pore water was collected, and the remaining sediment sample was weighed. The 10 ± 1 g-portions of the centrifuged sediment was weighed into a Falcon tube. Then the sediment was homogenized by stirring before taking an aliquot. The sample was extracted with 10 mL of acetonitrile by shaking for 20 min. After this, the sample was centrifuged for 5 min at 4000 rpm. The acetonitrile layer was decanted through filter paper into a 25 mL volumetric flask. The extraction was repeated with another 10 mL-portion of acetonitrile. The acetonitrile layers from the two extractions were combined and adjusted to a volume of 25 mL by addition of acetonitrile in a volumetric flask. The extract was transferred to a 25 mL EPA vial and dried with approximately 1 g of anhydrous sodium sulfate. The organic extracts were left to stand overnight in the freezer. A 15 mL-portion of the extract was taken with a 10 mL graduated pipet, concentrated using a TurboVap LV evaporation system (Caliper Life Sciences) and finally adjusted to a final volume of 1 mL in a volumetric flask. Into the sample, 10 μL of internal standard (hexachlorobenzene, HCB) was added. Then the sample was derivatized by adding 10 μL of TFAI (or alternatively BSTFA) and incubating the sample at 30 °C for 5 min. Finally the sample was analyzed by GC-MS/MS in the SRM mode.

GC-MS/MS Conditions in SRM Mode. GC-MS/MS conditions in SRM mode were optimized for TDG and TDGO derivatives, and energy of the collision cell at 1 to 30 eV was studied. On the basis of GC-MS spectra, the precursor ions m/z 200.0 and m/z 140.9 were selected for optimization of the TDG derivative and m/z 140.9 and m/z 69.0 for the TDGO derivative.

RESULTS AND DISCUSSION

Optimization of the Derivatization Reaction Conditions. *Selection of Organic Solvent.* The peak area was monitored, and based on correlations shown on Figure 4, acetonitrile, methylene chloride, and hexane resulted in the most rapid and efficient derivatization.

Selection of Time and Temperature. TDG derivatization with TFAI occurred almost immediately after the addition of the derivatization agent at any temperature ranging from 30 to 100 °C (Figure 5). A reaction time of 5 min was selected in order to be certain that the derivatization reaction was finished.

Water Influence. Data indicated 1% water content resulted in a drop in reaction efficiency and three compounds,

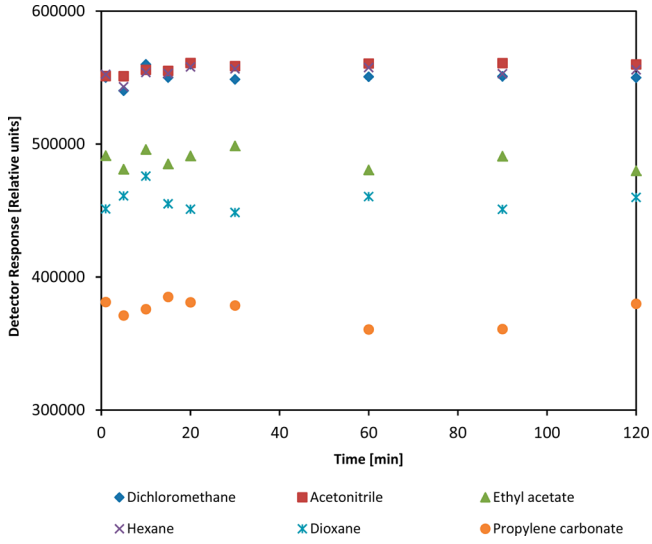

Figure 4. Influence of solvent type on thiodiglycol derivatization yield using TFAI.

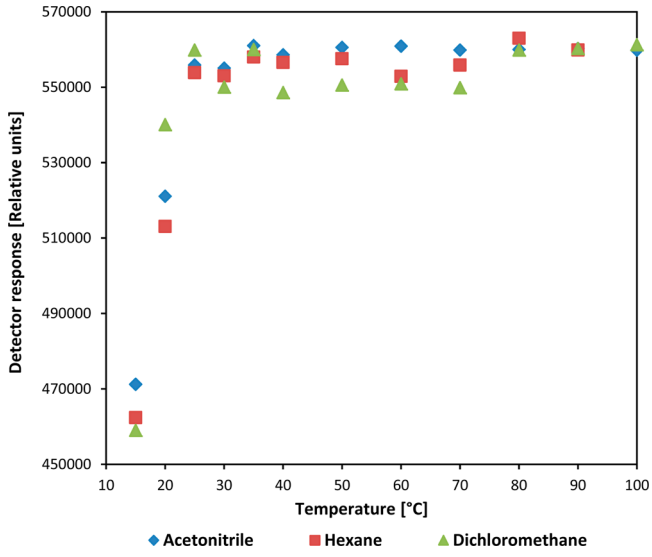

Figure 5. Effect of temperature on the efficiency of TDG derivatization reaction by TFAI.

thiodiglycol (approximately 10%), TDG-$(TFAI)_1$ (approximately 30%), and TDG-$(TFAI)_2$ (60%), were identified. At 5% water, only a partial derivatization was achieved, and TDG (60%), TDG-$(TFAI)_1$ (30%) and TDG-$(TFAI)_2$ (10%) were recovered. At 10% water, the derivatization reaction would not proceed and the mixture contained mostly TDG (85%) and completely derivatized TDG (~2%).

Direct Extraction from Water. The efficiency of direct extraction combined with derivatization was low in all cases, and the best efficiency (15%) was obtained using ethyl acetate, while hexane and methylene chloride showed lower efficiencies for the extraction-derivatization step at 10% and 2%, respectively. As the reaction gave 10% TDG-$(TFAI)_1$, another 10 μL portion of TFAI was added to the solution obtained from extraction which increased the efficiency to 25%.

Influence of Water on Stability of Derivative. Figure 6 shows that the derivative of TDG and TFAI becomes decomposed in the presence of low concentrations of water. The addition of 3 μL of water resulted in a 30% decrease in the

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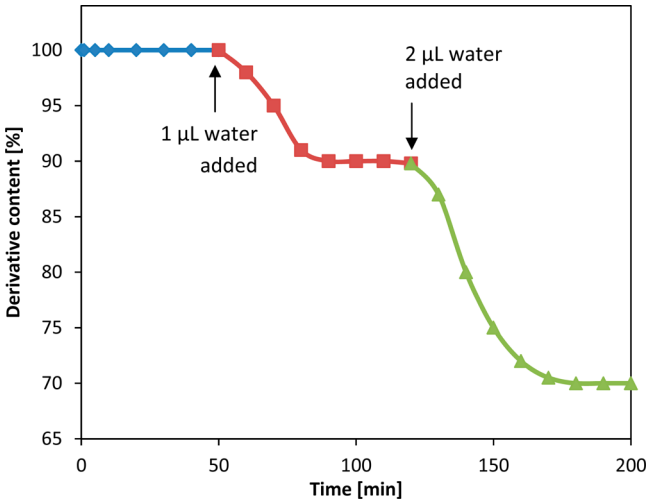

Figure 6. Effect of water on stability of the derivative obtained from derivatization of TDG with TFAI.

concentration of the derivation products (with respect to the initial concentration) over the next hour and the reaction appeared to stabilize after the water was consumed.

Stability of Derivative. Results of the experiment are presented in Figure 7. When TDG and TDGO were present

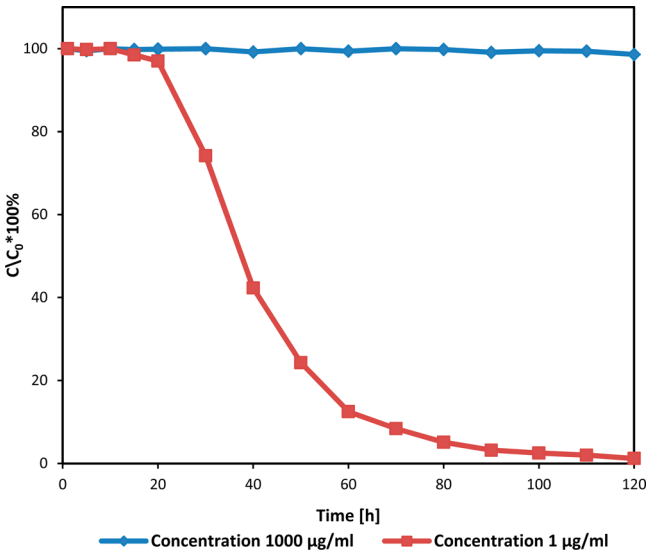

Figure 7. Effect of storage time on stability of the obtained TDG derivative for selected initial concentrations.

at high concentrations (1000 μg/mL), degradation was seen at 4 days, while dilute solutions (1 μg/mL) remained at the original concentration for ~10 h, after which a rapid drop in concentration was observed.

GC-MS/MS Conditions in SRM Mode. After measurement of the GC-MS/MS product ion spectra, the following transitions were selected for the TDG-TFAI derivative: m/z 200.0 to m/z 141.0, m/z 200.0 to m/z 86.0, and m/z 200.0 to m/z 85.0, while for the TDGO-TFAI derivative, transitions were m/z 140.9 to m/z 140.8, m/z 140.9 to m/z 68.8, and m/z 140.9 to m/z 47.0 were selected at a collision energy of 8 eV for TDG and 18 eV for TDG. To compare the data obtained in SRM analysis the following transitions were selected for the BSTFA derivative: for TDG 147 → 73, 116 → 101, 116 → 59 and collision cell energy 11 eV, for TDGO 117 → 73, 117 → 45, 73 → 45, and collision energy 12.5 eV.

Optimized Derivatization Procedure. Optimization studies and derivative stability tests demonstrated the TDG and TDGO derivatization reactions with TFAI occurred almost immediately following mixture of reagents at 30−80 °C, while below 30 °C, samples should be analyzed after 20 min as the reactions were slower (Figure 5). Moisture had an unfavorable effect on the derivatives which indicated samples should be protected against water and the solvents should be dried before use in order to achieve greater reaction efficiency. Derivatized concentrated TDG and TDGO solutions (concentration around 1 mg/mL) could be stored at room temperature for several days before analysis, while samples at low concentration levels (μg/mL) should be analyzed as soon as possible after the derivatization, as they degraded rapidly after ~10 h.

Using the procedure developed for derivatization with TFAI, derivatives of TDG, TDGO were obtained, for which retention indices and mass spectra were determined using a gas chromatograph coupled with a mass spectrometer, and the data are shown in Table 1.

Table 1. Mass Spectra Obtained during Analysis of TDG and TDGO after Derivatization by TFAI

	TDG		TDGO	
m/z	abundance	abundance %	abundance	abundance %
45	284 871	16		
47	153 440	9	62 400	5
59	282 501	16	116 698	10
60	297 525	17	105 029	9
69	1 588 141	91	1 123 925	95
75			131 486	11
76			63 592	5
85			142 740	12
86	1 029 056	59	59 363	5
97	96 550	6	54 091	5
113	165 407	9	64 370	5
141	1 750 425	100	1 177 240	100
200	637 077	36	67 926	6
201	190 030	11		

Comparison of Detection Methods for TFAI Derivatization of TDG. Calibration curves were produced by analyzing a series of TDG standards derivatized with TFAI using the optimized conditions described above for GC-AED (Figure 8) and GC-MS/MS in SRM mode (Figure 9). Also a calibration curve was produced for TDGO derivatives by GC-MS/MS in SRM mode (Figure 10). The limits of detection and quantification for TFAI-derivatized TDG were determined using the curve, which allowed comparison of both methods for analyses of compounds at high concentration above 100 ppb and low concentration up to 100 ppb.

On the basis of the calibration curve, the limits of detection and quantification for the TDG derivative by GC-AED were 1 ng/mL and 6 ng/mL, respectively. Detection and quantification limits for analysis of TDG derivatized with BSTFA were 61 ng/mL and 122 ng/mL, respectively.

Using GC-MS/MS for calibration curves, two ranges were notable: high concentrations (μg/mL order of magnitude) and low concentrations (ng/mL order of magnitude) were seen (Figure 9). These data suggested it was possible to determine the concentration of an unknown sample more precisely using

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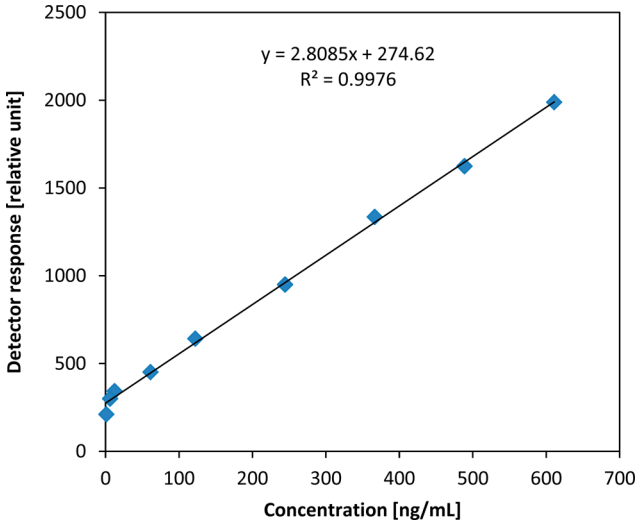

Figure 8. Calibration curve of TFAI-derivatized TDG. The signals were recorded on the sulfur channel (at 181 nm) of the atomic emission detector.

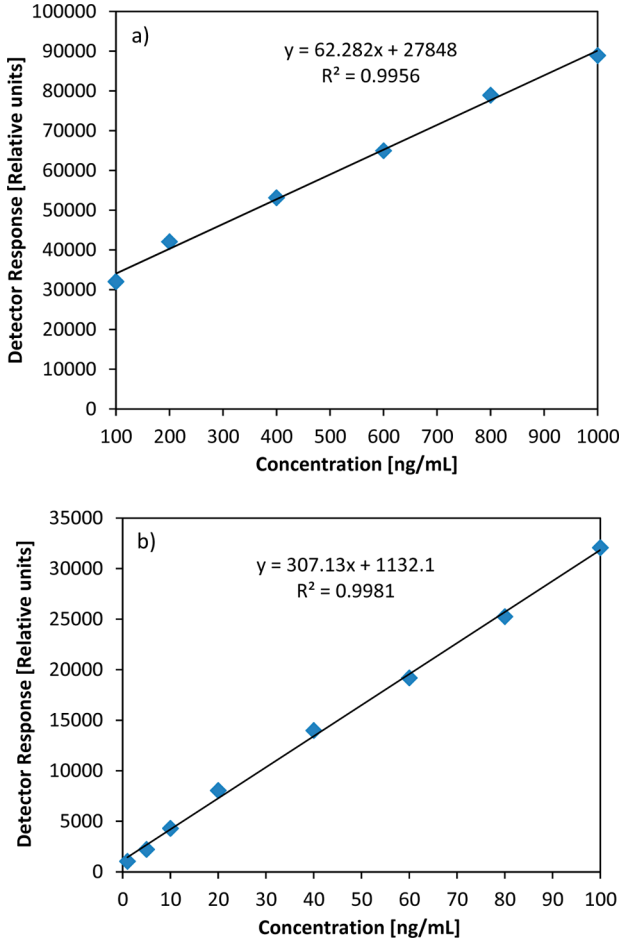

Figure 9. Calibration curves for TDG following TFAI derivatization using the GC-MS/MS in SRM mode (a) at high concentration and (b) at low concentration.

the independent calibration method, and the limit of detection was approximately 0.01 ng/mL and the limit of quantification was 0.3 ng/mL level (see Table 2).

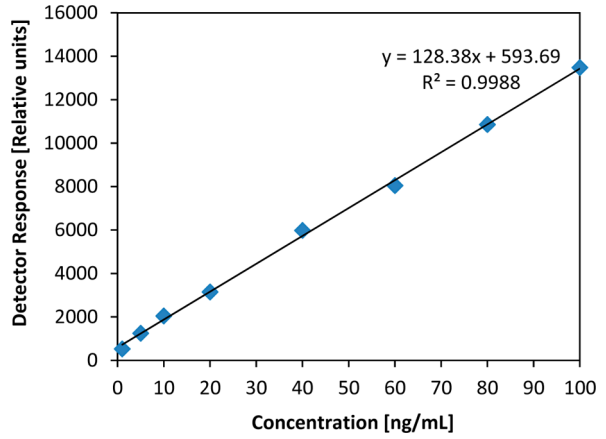

Figure 10. Calibration curve for high concentrations of TFAI-derivatized TDGO using GC-MS/MS in SRM mode.

Table 2. Comparison of Selected Parameters for TDG Derivatization with BSTFA and TFAI

factor	BSTFA	TFAI
reaction time	30 min at 60 °C	1 min at 30 °C
reaction temperature	at least 60 °C	at least 20 °C (however reaction goes slower than at 30 °C)
stability	2−3 days	1 week or more
water sensitivity	very high sensitivity	high sensitivity
limit of quantification	122 (GC-AED)	6 (GC-AED)
[ng/mL]	10 (GC-MS/MS)	0.3 (GC-MS/MS)
limit of detection	61 (GC-AED)	1 (GC-AED)
[ng/mL]	5 (GC-MS/MS)	0.01 (GC-MS/MS)

Comparison of Two Derivatization Methods during Analysis of Environmental Samples. These two derivatization methods (BSTFA and TFAI) were compared on real samples matrix. For this test, the sediment samples from the Baltic Sea were selected. The signal obtained from samples derivatizated by TFAI was higher than the signal from samples derivatizated by BSTFA, and chromatograms obtained during this test are shown in Figure 11. The use of GC-MS/MS allowed the acquisition of chromatograms of environmental samples with very low background for both derivatization methods. The LOQ of TDG and TDGO in sediment samples were determined, and these values were calculated for sediment dry weight. The LOQ for TDG and TDGO analyzed after derivatization by BSTFA were 1.57 and 6.29 μg/kg and for derivatization by TFAI were 0.25 μg/kg for TDG and 1.48 μg/kg for TDGO.

Results of Analysis of Environmental Samples. In order to test our method of derivatization, 20 sediment and core samples from the Baltic Sea were analyzed. The samples were collected during the CHEMSEA project (Chemical Munitions Search & Assesment). Three of those samples were positive for TDGO and four were positive for TDG; the results obtained during the analysis are shown in Table 3 and examples of chromatograms are in Figure 12.

CONCLUSIONS

TDG and TDGO derivatization with TFAI were rapid, occurring almost immediately after mixing of reagents if carried out at 30−80 °C. Small volumes of water in TFAI-derivatized

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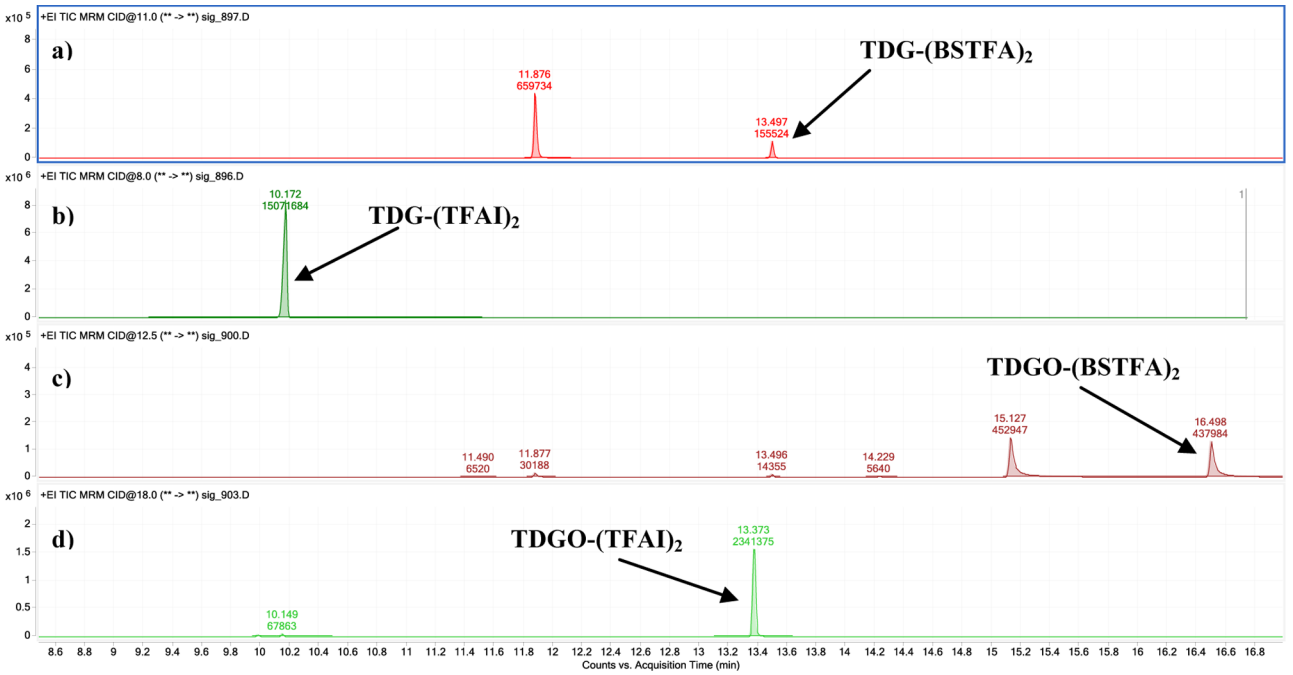

Figure 11. Chromatograms obtained during analysis of sediment samples spiked by TDG (a and b) and TDGO (c and d) and derivatized by BSTFA (a and c) and TFAI (b and d).

Table 3. Results from Analysis of Sediment Samples from Baltic Sea

internal	concn [μg/kg dw]		
sample code	TDG	TDGO	sampling area
Sediment 1	0	263.9	Gdańsk Deep
Sediment 4	0	196.8	Gdańsk Deep
Sediment 7	0	203.9	Gulf of Gdańsk
Slice 1	53.0	0	Gotland Deep
Slice 5	49.6	0	Gotland Deep
Slice 6	20.3	0	Gotland Deep
Slice 7	31.6	0	Gotland Deep

TDG samples had an unfavorable effect on derivatization, and water content at 0.3% (v/v) reduced the initial derivatization product concentration by approximately 30%. Using GC-MS/MS to analyze TDG derivatized with TFAI allowed detection at ~1 ng/mL with a limit of detection at ~0.01 ng/mL. The linear dynamic range of that method for TDG determination was 0.3−100 ng/mL and a second range from 100 to 1000 ng/mL. Trifluoroacetyl derivatives formed by TDG and TDGO derivatization were stable in concentrated solutions (close to 1 mg/mL) for 4 days at room temperature, while derivatives in diluted solutions (1 μg/mL) started to degrade within 10 h, most likely due to the presence of small quantities of water in the solution after derivatization.

The limit of detection for TDG was decreased by 2 orders of magnitude if TFAI (instead of silyl derivatizing agents BSTFA or MTBSTFA) was used for derivatization. Derivatization of TDG and TDGO with TFAI occurred within 1 min above 20

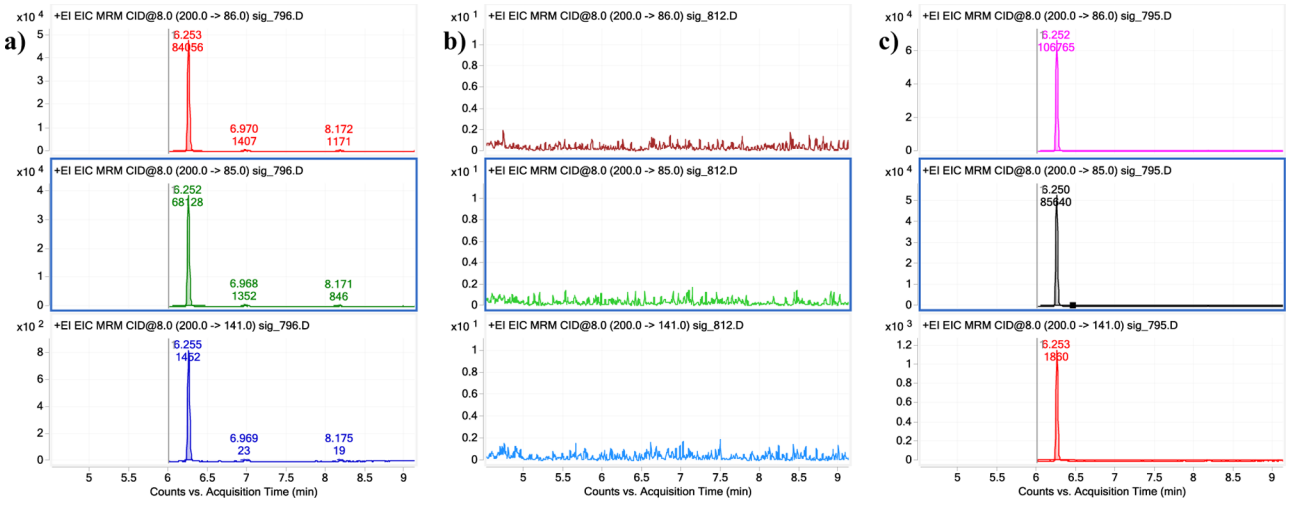

Figure 12. SRM chromatograms, with transition 200 → 86, 200 → 85, and 200 → 141, obtained during analysis of (a) TDG-$(TFAI)_2$ derivative reference, (b) blank sample, and (c) sediment sample from the Baltic Sea.

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°C, while the use of BSTFA as a derivatizing agent required higher temperatures (at least 60 °C) and longer reaction times (30−60 min). These data suggested TFAI derivatization could be more applicable when limited time and resources are available (field conditions).

Derivatization with TFAI (over BSTFA) for TDG analysis by GC-AED was shown, and data indicated the reaction was more sensitive, was faster, and proceeded under mild conditions compared to BSTFA derivatization (the reaction goes slowly at 70 °C). Both derivatives had similar sensitivities to water, but BSTFA derivatization was more sensitive and less stable over time than TFAI derivatization.

Derivatization with TFAI was used for analysis of sediment samples from the Baltic Sea. The target chemicals were degradation products of sulfur mustard, including TDG and TDGO. This method was successfully used for this analysis, and seven samples have rendered positive results. Comparison of derivatization by BSTFA and TFAI on real samples shows that the signal obtained from the TFAI derivative is much higher than signal obtained from the BSTFA derivative. Also, the analysis of real samples in the SRM mode allowed the acquisition of chromatograms with background on a very low level.

In case of TDGO analysis, the use of TFAI as a derivatization agent allows one to obtain the results with very low amounts of the artifacts, and the only product of derivatization is TDGO derivative. TFAI has three major advantages in comparison to BSTFA, the first is much higher sensitvity, the second is a very clean background, and the last one are very mild conditions of derivatization.

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Notes

The authors declare no competing financial interest.

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