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Journal of Chromatography A, 1061 (2004) 235–241
JOURNAL OF CHROMATOGRAPHY A
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Short communication

Determination of thiodiglycol, a mustard gas hydrolysis product by gas chromatography–mass spectrometry after *tert*-butyldimethylsilylation

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Received 25 August 2004; received in revised form 25 October 2004; accepted 27 October 2004

Abstract

A method for determining thiodiglycol (TDG), a mustard gas hydrolysis product in water, serum and urine samples using gas chromatography–mass spectrometry (GC–MS) after *tert*-butyldimethylsilylation (TBDMS) is described. Quantitation of TDG was performed by measuring the respective peak area on the extracted ion chromatogram of *m/z* 293, using an internal standard, the TDG homologue, thiodipropanol, peak area of which was measured as *m/z* 321. The presence of salts in the sample solution not only suppressed the loss of TDG by vaporization during the evaporation of water, but also facilitated the rate of production of di-silylated derivative, bis(*tert*-butyldimethylsilyoxylethyl)sulfide (TDG-$(TBDMS)_2$). Under the pretreatment conditions used, in which 0.5 ml of water sample supplemented with 100 μM potassium chloride was evaporated to dryness under reduced pressure, followed by reaction with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide at 60 °C for 1 h, TDG-$(TBDMS)_2$ was reproducibly detected with about a 55% recovery and a limit of detection (LOD, scan mode, S/N = 3) of 5.4 ng/ml. TDG was also determined by GC–MS from a 0.5 ml serum sample (after perchloric acid deproteinization) and from a 0.1 ml urine sample, after TBDMS derivatization. The LOD was determined to be 7.0 and 110 ng/ml for serum and urine, respectively.
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Keywords: Derivatization, GC; Thiodiglycol

1. Introduction

Mustard gas (HD, bis(2-chloroethyl)sulfide) is a blistering agent [1], that was extensively used in World War I and II, and continued to be produced for warfare [2]. HD was also used in the Iran–Iraq conflict in the 1980s [3], and use of chemical warfare agents (CWA) is now prohibited by the Chemical Weapons Convention on the prohibition of the development, production, stockpile and use of chemical weapons and on destruction [4]. In 1995, AUM Shinrikyo caused a sarin gas attack in the Tokyo subway system against defenseless people [5]. This real incident points to the great threat of chemical warfare terrorism. In addition, the disarmament of stockpiled chemical weapons is now an urgent issue, and, as a result, the disposition of chemical weapons abandoned in China by the former Japanese military forces just after World War II have become serious social and diplomatic problems. Blistering, vomiting, blood agents and tear gases produced by the former Japanese military forces were also left behind in Japan, and recently have been excavated from former Japanese military force facilities during land excavations [6]. Therefore, the detection and identification of blistering agents is important in terms of potentially dangerous terrorist situations and validating their presence and possible exposure.

Concerning the analysis of CWA including HD, various methods have been reviewed [7,8], and a recommended protocol from sample treatment to final instrumental analysis is available [9]. HD is easily decomposed both in in vivo and in the environment [2]. In water, HD is hydrolyzed to thiodiglycol (TDG), a stable nonvolatile and nontoxic compound [10]. Therefore, instead of identifying HD, the determination of TDG should provide indirect proof of the existence of HD. Chromatographic analyses of decomposition products

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doi:10.1016/j.chroma.2004.10.087

of CWA including TDG, after derivatization [11], have been reviewed. TDG has been analyzed by liquid chromatography (LC)–mass spectrometry (MS) [12], LC coupled with flame photometric detection [13] and capillary electrophoresis [14]. TDG can be derivatized by pentafluorobenzoylation [15], heptafluorobutylation [16] and trimethylsilylation [17], and analyzed by gas chromatography (GC)–MS.

Our laboratory has adopted *tert*-butyldimethylsilylation (TBDMS) derivatization [18] for the GC–MS analysis of the nerve gas hydrolysis products [5], methylphosphonic acid (MPA) and alkylmethylphosphonic acids (RMPA), and developed pretreatment methods for soils and biological samples [19–21]. If the TBDMS derivatization technique is adequate for the GC–MS analysis of TDG, a GC–MS method after TBDMS derivatization would be advantageous for the simultaneous determination of nerve gas and HD hydrolysis products. Concerning environmental samples, Tomkins and Sega [22] developed a TBDMS GC–MS method for groundwater samples. Adopting a tandem solid phase extraction using the octadecylsilica support and a hydrophobic carbonaceous sorbent with a large surface area, and pyridine, to retain the TDG during the extraction solvent evaporation, they attained a limit of detection (LOD) between 4 and 16 ng/ml but rather poor extraction recovery of 23%. Black et al. reported on the analysis and detection of CWA and related compounds in various environmental samples taken from the Kurdish village of Birjinni, which had been attacked by the Iraq military forces [23]. They detected TDG and the other decomposition compounds by TBDMS GC–MS from aqueous extracts of samples, but did not report on the precision of the method.

Here, we report on the improvement of a TBDMS derivatization method for the GC–MS analysis of TDG from water, serum and urine samples, utilizing salt addition effect.

2. Experimental

2.1. Reagents

TDG and thiodipropanol (TDP) were obtained from Sigma–Aldrich chemicals (Milwaukee, WI, USA). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL, USA). Stock solutions, TDG and TDP, were prepared in acetonitrile (10 mg/ml) and stored in tightly capped glass vials at −20 °C, and were used within 1 month. Working solutions (100 μg/ml) were prepared by diluting the stock solution with additional acetonitrile.

Human serum sample was obtained from the Tokyo Metropolitan Police Hospital (Iidabashi, Tokyo, Japan) as outdated transfusion serum. Human urine sample was collected from a volunteer at our institute.

2.2. tert-Butyldimethylsilylation

TBDMS derivatization was performed as described previously [21]. The aqueous sample solution was taken to dryness in a 1-ml screw-capped glass vial (Nichiden Rika Garasu, type MV-7, Tokyo, Japan), as described in Section 2.4. Into the vial, 50-μl of MTBSTFA and 50 μl of an acetonitrile solution, which contained 25 μg/ml of TDP [internal standard (IS) to correct for the derivatization] and 25 μg/ml of nonadecane [C_{19}, IS to correct for the GC injection volume] were added, and the vial was sealed with Teflon-cap, homogenized by sonication for 5 min and incubated at 60 °C for 1 h. For the standard, into a new vial, a 50-μl volume of MTBSTFA and 50 μl of an acetonitrile solution, which contained an appropriate amount of TDG and 25 μg/ml of TDP and 25 μg/ml of C_{19}, were added. The vial was then sealed, homogenized and incubated at 60 °C for 1 h. A 1-μl volume of the mixture was then applied to the GC–MS system, as described below.

2.3. Gas chromatography–mass spectrometry

The GC–MS system consisted of an HP 6890 gas chromatograph combined with an HP 5973 quadrupole mass spectrometer (Agilent Technology, Tokyo, Japan). GC was performed on a DB5-MS capillary column (30 m × 0.25 mm i.d., 0.25 μm thickness, J&W Scientific, Folson, CA). The carrier-gas (helium) flow-rate and split ratio were adjusted to 0.8 ml/min and 30, respectively. The injection port and transfer line were maintained at 250 and 280 °C, respectively. The temperature program for the separation was as follows, initial temperature, 90 °C (1 min hold), then a ramp to 290 °C at 20 °C/min (5 min hold). Mass spectrometric acquisition was started 5 min after sample injection. For the qualitative and quantitative analysis, an electron ionization (EI, ionization energy, 70 eV; ionization current, 34.6 μA) was used as the ionization mode. The ion source and quadrupole analyzer were maintained at 230 and 106 °C, respectively. The data acquisition mass range was 50–550, and sampling was 0.8 scan/s. The extracted ion chromatograms were obtained at *m*/*z* 293 for the TDG derivative, *m*/*z* 321 for the TDP derivative, and *m*/*z* 268 for C_{19}. For the structural confirmation, chemical ionization (CI, ionization energy 105 eV, ionization current 237 μA) was used. Reagent gas used was methane (1.5×10^{-5} Torr). The ion source and quadrupole analyzer were maintained at 250 and 106 °C, respectively. The data acquisition mass range was 100–550, and sampling was 0.8 scan/s.

2.4. Sample pretreatment

A 0.5 ml volume of an aqueous sample was spiked with 5–25 μl of an acetonitrile solution of TDG, and concentrated to dryness under reduced pressure at 50 °C on a Model EC-57CS centrifugal evaporator (Sakuma Seisakusho, Tokyo, Japan) in a 1-ml screw-capped glass vial. Alternatively, the concentrated solution was frozen in liquid nitrogen in a glass vial, and dried on a centrifugal evaporator (freeze-dry).

A 0.5 ml volume of human serum was spiked with 5–25 μl of an acetonitrile solution of TDG, and combined with 2 ml of 4% perchloric acid. After vortexing for 1 min, the mixture

was centrifuged at 1500 × *g* for 5 min, and a 0.5 ml aliquot of the resulting supernatant was neutralized with potassium carbonate. The resulting solution was again centrifuged, and the supernatant was dried by the above-mentioned methods. A 0.1 ml volume of human urine was spiked with 5–10 μl of an acetonitrile solution of TDG, and dried by the above-mentioned methods.

3. Results

3.1. Gas chromatography–mass spectrometry of tert-butyldimethylsilylated thiodiglycol

A 50 μl volume of an acetonitrile solution containing 1 mg/ml of TDG, 1 mg/ml of TDP and 0.2 mg/ml of C_{19} was combined with 50 μl of MTBSTFA, and incubated at 60 °C for 1 h, and analyzed by GC–MS. Fig. 1 shows the total ion chromatogram and extracted ion chromatograms. The TBDMS derivative of TDG was eluted at 8.9 min, and gave characteristic EI mass spectrum of di-*tert*-butyldimethylsilylated TDG (TDG-$(TBDMS)_2$, Fig. 1E), in which a des-*tert*-butyl molecular ion ($[M–(CH_3)_3C]^+$, *m/z* 293) was observed in addition to a TBDMS derivative specific fragment ion (*m/z* 147). The CI mass spectrum of the TBDMS derivative of TDG gave ethyl adduct ion ($[M+C_2H_5]^+$, *m/z* 379), des-methyl molecular ion ($[M–CH_3]^+$, *m/z* 335), $[M–(CH_3)_3C]^+$ (*m/z* 293) and des-*tert*-butyldimethylsilyloxy ion ($[M–(CH_3)_3CSi(CH_3)_2O]^+$, *m/z* 219) (data not shown). The EI and CI mass spectra of the TDG-$(TBDMS)_2$ is consistent with those of the reference report [23].

The TBDMS derivative of TDP, a homologue of TDG, was eluted at 9.8 min, and gave a characteristic EI mass spectrum of di-*tert*-butyldimethylsilylated TDP (TDP-$(TBDMS)_2$, Fig. 1F), where a $[M–(CH_3)_3C]^+$, (*m/z* 321) was observed in addition to a fragment ion of *m/z* 147. The CI mass spectrum of the TBDMS derivative of TDP gave $[M+C_2H_5]^+$ (*m/z* 407), $[M–CH_3]^+$ (*m/z* 363), $[M–(CH_3)_3C]^+$ (*m/z* 321) and $[M–(CH_3)_3CSi(CH_3)_2O]^+$ (*m/z* 247) (data not shown). On the extracted ion chromatogram of *m/z* 293 in the EI mode, only one peak appeared (Fig. 1B), and, similarly only one peak appeared on the extracted ion chromatogram of *m/z* 321 (Fig. 1D). On the extracted ion chromatogram of *m/z* 268,

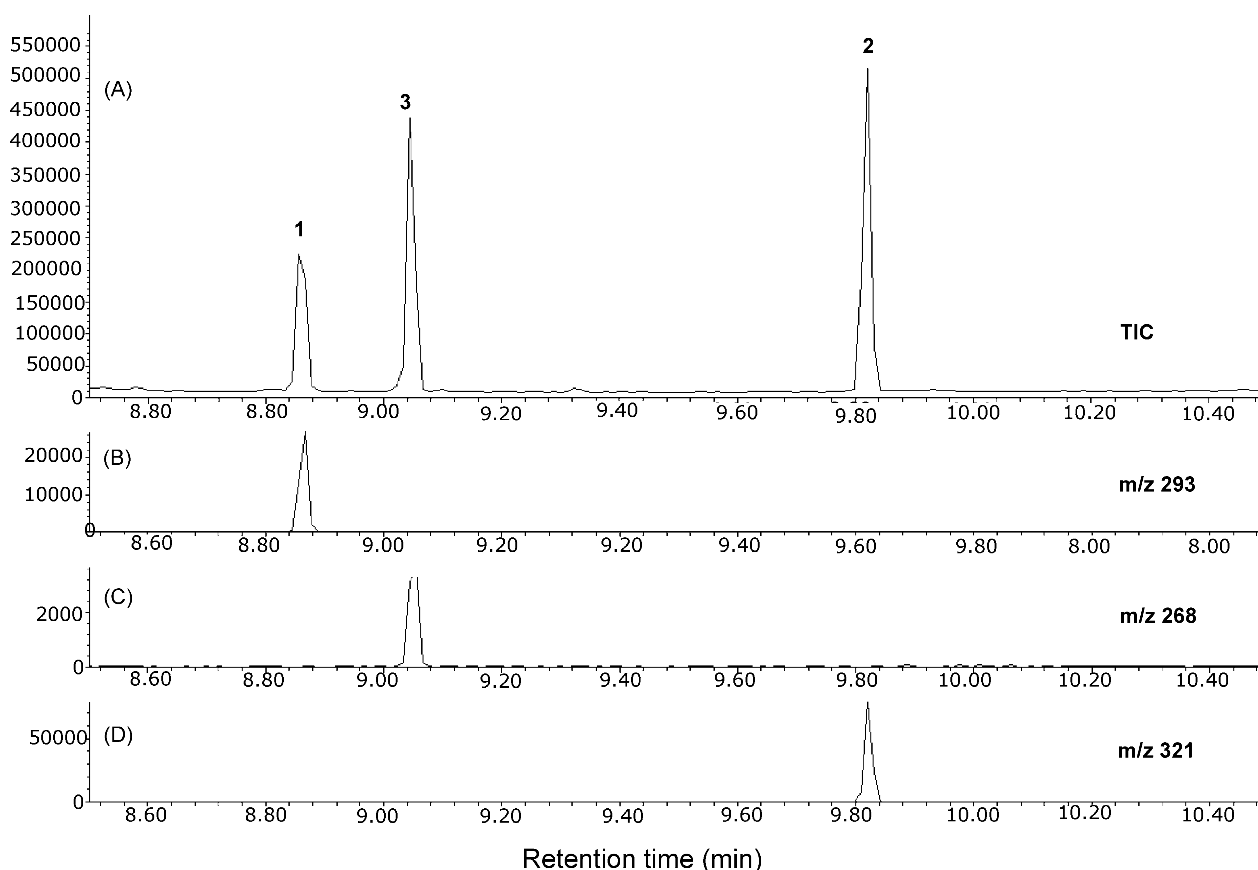

Fig. 1. Gas chromatography–mass spectrometry of *tert*-butyldimethylsilyl derivatives of thiodiglycol and thiodipropanol. A 50 μl acetonitrile solution containing 1 mg/ml of TDG (1), 1 mg/ml of TDP (2), 0.2 mg/ml of nonadecane (C_{19}, 3) and 50 μl of MTBSTFA was incubated at 60 °C for 1 h, and the resulting reaction mixture was subjected to GC–MS. (A) Total ion chromatogram (TIC); (B)–(D) extracted ion chromatogram of *m/z* 293 (B), *m/z* 268 (C) and *m/z* 321 (D); (E) mass spectrum of the peak 1 in Fig. 1A (TDG-$(TBDMS)_2$); (F) mass spectrum of peak 2 in Fig. 1A (TDP-$(TBDMS)_2$).

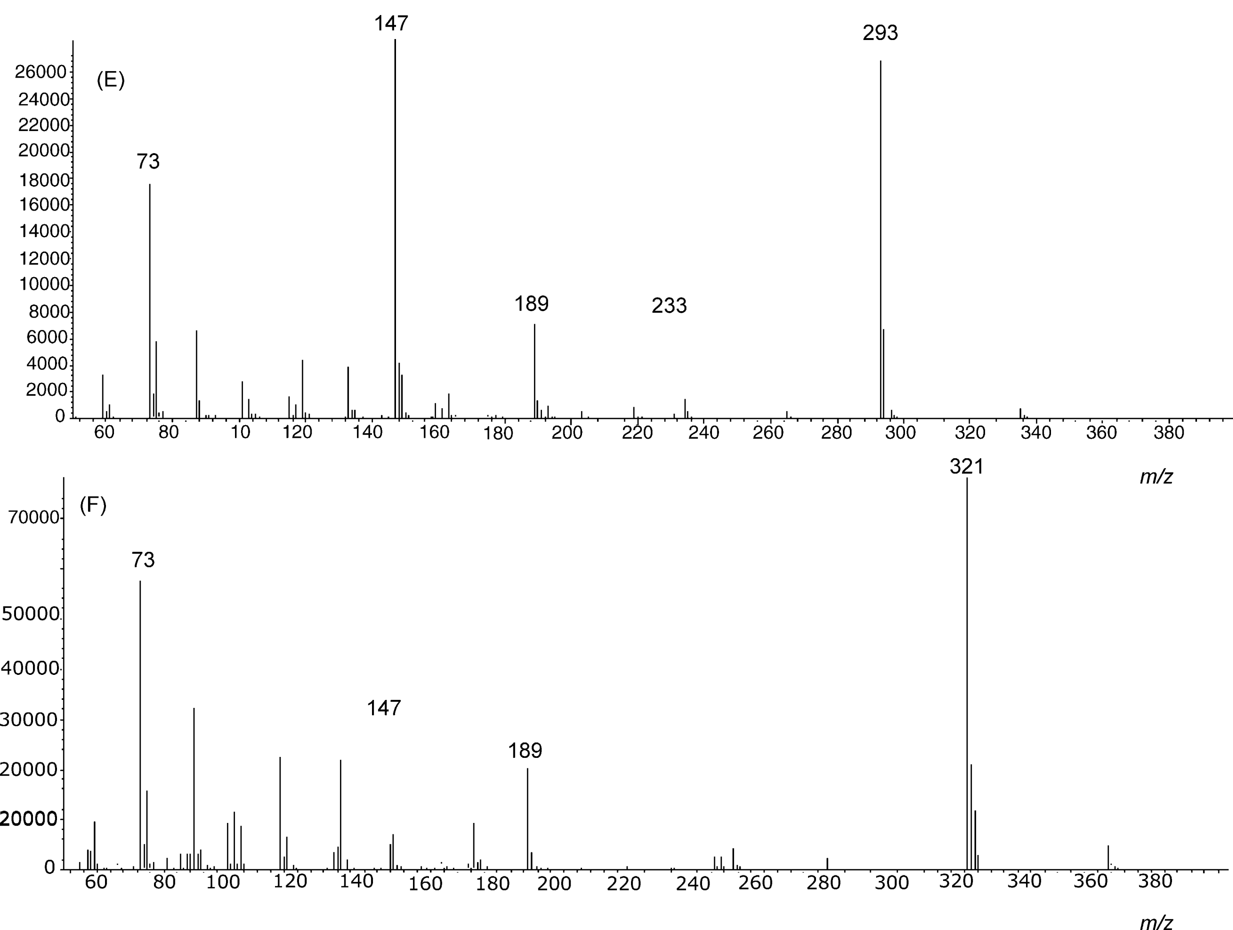

Fig. 1. (*Continued*).

which was a molecular ion of C_{19}, only one peak appeared at 9.1 min (Fig. 1C).

In the early stage of the reaction of TDG with MTBSTFA, monosubstituted derivative of TDG (2-*tert*-butyldimethylsilyloxyethyl(2-hydroxyethyl)sulfide, TDG-TBDMS) was also found, which eluted at 7.1 min. EI mass spectrum of the TDG-TBDMS gave $[M-(CH_3)_3C]^+$ (*m/z* 179) and $[M-(CH_3)_3C-OH + H]^+$ (*m/z* 163) (data not shown). CI mass spectrum of the TDG-TBDMS gave $[M+C_2H_5]^+$ (*m/z* 265), $[M-OH]^+$ (*m/z* 219) and $[M-(CH_3)_3C]^+$ (*m/z* 179) (data not shown).

For a quantitative estimation, the areas under the peaks of the TDG-$(TBDMS)_2$, TDP-$(TBDMS)_2$ and nonadecane on the respective extracted ion chromatograms were measured.

3.2. Derivatization efficiency of thiodiglycol with MTBSTFA

During the derivatization of TDG with MTBSTFA, the amount of TDG-$(TBDMS)_2$ increased linearly with increasing the reaction time, and saturation was not observed within 1 h, a period of time, that led the saturation of TBDMS derivative produced from MPA and RMPA [19]. The increase in TDG-$(TBDMS)_2$ was also observed, even when the reaction was performed at room temperature. The peak of TDP-TBDMS appeared at the early reaction stage and grew to the maximum level at about 5 h, and completely disappeared after 1 day incubation (data not shown).

As shown in Table 1, the addition of a salt to the derivatization reaction mixture dramatically increased the amounts of TDG-$(TBDMS)_2$ and TDP-$(TBDMS)_2$. Potassium chloride and potassium perchlorate increased their amount by 70–90 fold, compared to a salt blank. Sodium chloride, magnesium chloride and sodium sulfate increased the yield by 10–50 fold. But manganese chloride had no effect on the amounts produced. The increase in efficiency was similar for both TDG and TDP. In the presence of 500 μM KCl, the amount of TDG-$(TBDMS)_2$ and TDP-$(TBDMS)_2$ increased rapidly and then reached a constant value for times exceeding 1 h, in contrast to those without KCl, where the amounts of TDG-$(TBDMS)_2$ and TDP-$(TBDMS)_2$ increased nearly linearly for periods of up to 7 h, and then became saturated (Fig. 2). The peak of TDP-TBDMS appeared at the early reaction stage and grew to the maximum level at about 1 h, and completely disappeared after several hour incubation (data not shown). The effect of KCl on increasing amounts of TDG-$(TBDMS)_2$

Table 1
Effect of salt on the yield of the *tert*-butyldimethylsilylated derivatives of thiodiglycol and thiodipropanol

Salt	Amount (mmol)	TDG-(TBDMS)$_2$/C$_{19}$	TDP-(TBDMS)$_2$/C$_{19}$	TDG-(TBDMS)$_2$/TDP-(TBDMS)$_2$
None	–	0.18 ± 0.04	3.1 ± 0.8	0.061 ± 0.009
KCl	0.3	12 ± 0.2	210 ± 6	0.059 ± 0.002
$KClO_4$	0.16	16 ± 0.4	280 ± 5	0.058 ± 0.002
NaCl	0.3	9.3 ± 1.2	84 ± 19	0.11 ± 0.01
$MgCl_2$	0.31	2.3 ± 0.4	62 ± 10	0.037 ± 0.002
$MnCl_2$	0.31	0.14 ± 0.18	0.26 ± 0.19	0.41 ± 0.22
Na_2SO_4	0.15	5.2 ± 0.7	39 ± 11	0.14 ± 0.02

Into a glass vial containing an appropriate amount of salt, 50 μl of an acetonitrile solution containing 2 μg/ml of TDG, 25 μg/ml of TDP, 25 μg/ml of C$_{19}$ and 50 μl of MTBSTFA was added, and incubated at 60 °C for 1 h, and the resulting reaction mixture was subjected to GC–MS. The peak areas of TDG-(TBDMS)$_2$, TDP-(TBDMS)$_2$ and C$_{19}$ on the extracted ion chromatogram of *m/z* 293, 321 and 268 were measured. The ratio of the peak area of the analyte to that of C$_{19}$ was presented. Each value represents an average ± standard deviation of three trials.

and TDP-(TBDMS)$_2$ was observed even with a minute amount present (100 μM), and saturated amounts of derivatives were obtained after 1 h with more than 300 μM KCl (Fig. 3).

3.3. Quantitation of thiodiglycol in an aqueous sample

Under the conditions used to evaporate aqueous solutions of TDG, the recovery of TDG was low (below 10%), indicating loss of TDG by evaporation. After evaporation by freeze drying without and with 500 μM KCl, a sample, including the appropriate amount of TDG, was analyzed by TBDMS GC–MS. The size of the TDG-(TBDMS)$_2$ peak fluctuated due to the lack of reproducibility of the derivatization reaction, and could not be improved even by using an IS (C$_{19}$). This result is different from results obtained for MPA and RMPA [19,21]. In contrast, reproducibility could be achieved by the use of an IS (TDP), and the calibration curves (peak area ration of TDG-(TBDMS)$_2$ to TDP-(TBDMS)$_2$ versus TDG concentration) were linear from 0 to 500 ng/ml without KCl ($y = 0.0018x - 0.042$, $\gamma^2 = 0.98$ (TDG addition after evaporation); $y = 0.00067x + 0.0099$, $\gamma^2 = 0.98$ (TDG addition prior to evaporation)) and with KCl ($y = 0.00031x - 0.000081$, $\gamma^2 = 0.998$ (TDG addition before evaporation); $y = 0.00017x + 0.0034$, $\gamma^2 = 0.99$ (TDG addition prior to evaporation)). The recoveries of TDG from aqueous samples were determined to be 36% without KCl and 55% with KCl, respectively, calculated from a comparison of the curve slopes. The coefficients of variation ($n = 3$) at 250 ng/ml were 21% without KCl and 10% with KCl, respectively. The limit of detection (LOD) measured by the scan mode was determined to be 5.4 ng/ml (S/N = 3).

3.4. Quantitation of thiodiglycol in blood serum and urine samples

TDG in serum can be easily extracted and deproteinized by treatment with a strong acid, and perchloric acid is

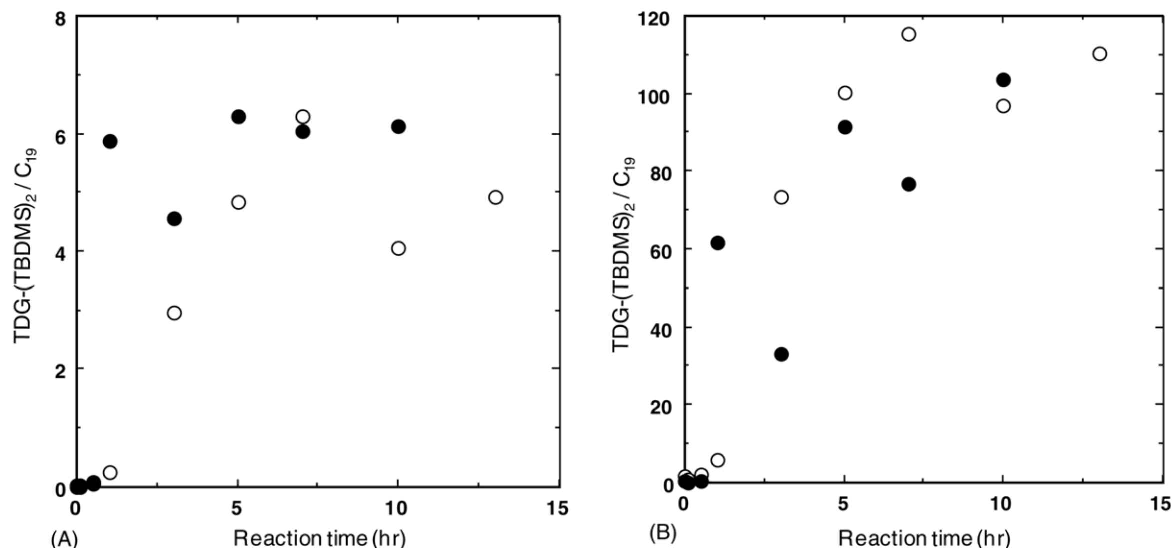

Fig. 2. Time course of the production of TBDMS derivative of TDG. A 50 μl acetonitrile solution containing 2 μg/ml of TDG, 25 μg/ml of TDP, 25 μg/ml of C$_{19}$ and 50 μl of MTBSTFA with (closed circles) and without (open circles) KCl residue (final 500 μM) was reacted at 60 °C for an appropriate time, and the resulting reaction mixture was subjected to GC–MS. The peak areas of TDG-(TBDMS)$_2$ (A), TDP-(TBDMS)$_2$ (B) and C$_{19}$ on the extracted ion chromatogram of *m/z* 293, 321 and 268 were measured, and the ratio of the peak area of TBDMS derivative to that of C$_{19}$ was plotted against incubation time.

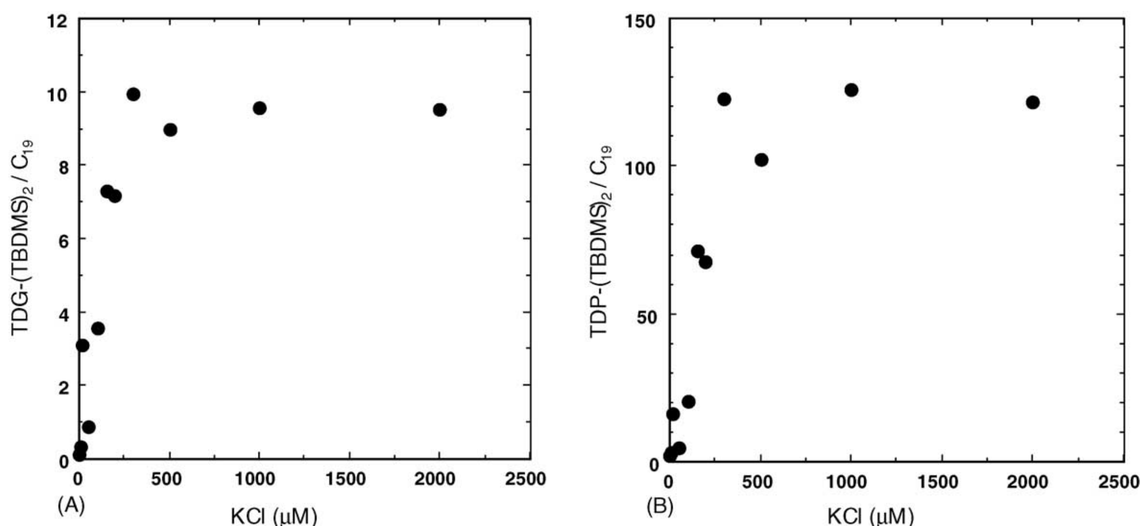

Fig. 3. Effect of potassium chloride on the production of TBDMS derivative of TDG. Into a glass vial containing KCl, 50 μl of an acetonitrile solution containing 2 μg/ml of TDG, 25 μg/ml of TDP, 25 μg/ml of C_{19} and 50 μl of MTBSTFA was added, and the solution was incubated at 60 °C for 1 h, and the resulting reaction mixture subjected to GC–MS. The peak areas of TDG-$(TBDMS)_2$ (A), TDP-$(TBDMS)_2$ (B) and C_{19} on the extracted ion chromatogram of *m/z* 293, 321 and 268 were measured, and the ratio of the peak area of TBDMS derivative to that of C_{19} was plotted against the KCl concentration in the reaction mixture.

typically used for this purpose. Perchloric acid for the deproteinization of serum samples, and the usage of this agent improved the recovery of TDG during evaporation procedure, and also facilitated the derivatization reaction, as a matrix effect (Table 1). Combined with freeze-drying evaporation, a serum sample, including the appropriate amount of TDG, could be analyzed by TBDMS GC–MS. The peak area for TDG-$(TBDMS)_2$ also fluctuated due to the lack of reproducibility of the derivatization, and was not improved even by correction with an IS (C_{19}). By the correction with TDP, the calibration curves were linear from 0 to 500 ng/ml ($y = 0.000092x + 0.0014$, $\gamma^2 = 0.999$ (TDG addition after evaporation); $y = 0.000058x + 0.0010$, $\gamma^2 = 0.99$ (TDG addition prior to evaporation)). The coefficient of variation ($n = 3$) at 250 μg/ml was 16%. The LOD measured by the scan mode was determined to be 7.0 ng/ml (S/N = 3). The recovery of TDG from the serum sample was determined to be 64%, calculated from a comparison of the curve slopes. From the intercept of the calibration curve at the *x*-axis, the blank value of TDG in the serum sample was calculated to be 18 ng/ml.

TDG in urine can be directly analyzed by TBDMS GC–MS after evaporation. Various salts in the urine sample may improve the recovery of TDG during the evaporation procedure, and also facilitate the derivatization reaction. The peak area of the TBDMS derivative of TDG also fluctuated, and was not improved even by the correction with an IS (C_{19}). After correction with TDP, the calibration curves were linear from 0 to 2 μg/ml ($y = 0.000034x + 0.0033$, $\gamma^2 = 0.94$ (TDG addition after evaporation); $y = 0.000027x + 0.00040$, $\gamma^2 = 0.98$ (TDG addition prior to evaporation)). The coefficient of variation ($n = 3$) at 1 μg/ml was 25%. The LOD measured by the scan mode was determined to be 110 ng/ml (S/N = 3). The recovery of TDG from the urine sample was determined to be 80%, calculated from a comparison of the curve slopes.

4. Discussion

TDG is an analytical target compound for verifying the use of and exposure to HD. The method described here is simple, compared to the method of Tomkins and Sega, and gives almost similar LOD value (5.4 ng/ml) and a higher recovery (55%) from aqueous samples. For the determination of MPA and RMPA using TBDMS GC–MS, we used C_{19} or anthracene as IS [19,21]. The findings herein indicate that, in contrast to the large fluctuation in derivatization yields with the correction with C_{19}, the correction of the yield values with the IS of TDP led to an improved reproducibility. Early in this research, we experienced no saturation in the TDG-$(TBDMS)_2$ under the same TBDMS derivatization conditions as was used in the analysis of RMPA and MPA (60 °C, 1 h). Moreover, the recovery was low during the evaporation step. In contrast, a high derivatization yield was obtained for the serum sample after a perchloric acid deproteinization procedure, indicating a positive matrix effect for the salt. This phenomenon is in contrast with the derivatization of MPA and RMPA, which was suppressed in the presence of salts [19]. The inclusion of KCl facilitated the efficiency of TBDMS derivatization, enabling rapid (1 h) and reproducible production of TDG-$(TBDMS)_2$. It is possibly postulated that the second derivatization reaction of the monosubstituted derivative (TDG-TBDMS) could be efficiently proceeded in the presence of the salt. Concerning the salt species, this efficient effect was observed for potassium and sodium (Table 1). Moreover, KCl functions to suppress the loss of TDG during the evaporation process. The exact mechanism of this salt

effect is unknown at this point, but this metal cation may interact with TDG molecules, resulting both in the fixation on the glass surface on the vial and in increasing the reactivity of TDG against MTBSTFA.

For verification of HD exposure, it is important to determine HD or related compounds in biological samples. The direct detection of HD is impossible because of the high reactivity of HD with the biomolecules and water. Recently, Noort et al. developed a method for determining HD adducts with biological macromolecules [24]. Their method, however, is time-consuming and labor-intensive. Instead, the determination of TDG in blood and urine samples is an orthodox standard method, and derivatization GC–MS techniques are in widespread use. Black and Read [25] developed GC-negative CI MS. Adopting a combination of solid phase extraction using a Clin Elut tube and octadecylsilica cartridge, and pentafluorobenzoylation derivatization, they attained an LOD of 1 ng/ml. They also developed a GC–MS method for HD metabolites and thiodiglycol sulphoxide after pentafluorobenzoylation [15,26]. Wils et al. [27] developed a headspace GC method after the conversion of TDG to HD by an HCl reaction, and attained a LOD of a few ng/ml in urine. In blood and urine, trace levels of TDG can be detected, and the levels were reported to be 9 ± 5 ng/ml in blood from normal subjects [25] and less than 1 ng/ml [25] and 5 ± 3 ng/ml [28] in urine from normal subjects. An increased level of TDG in urine from the victims attacked with HD has been reported [28]. The LOD for the method established in this paper was 7.0 ng/ml for serum. Thus, it is possible to discriminate the increased level of TDG in serum due to HD exposure. The blank TDG level in the serum sample observed in this experiment (18 ng/ml) is consistent with the normal blood level reported by Black and Read [25]. In contrast, the LOD in urine (110 ng/ml) is not sufficient to discriminate the background urine level. Moreover, the within-day repeatability (CV: 25%) was not satisfactory. This is probably due to the absence of a cleanup pretreatment, and many components present in a normal urine sample, substances themselves or derivatives produced during the TBDMS reaction, interfere with the detection of the TBDMS derivative of TDG. Boyer et al. [29], who developed a sensitive GC tandem MS method using time-consuming and labor-intensive procedure and stable isotope standard (LOD: 0.5 ng/ml) reported that pretreatment procedure of urine samples by deglucuronidation and acidic $TiCl_3$ reduction was necessary to correctly estimate the level of the urine TDG. We are currently designing a method for improving the detection sensitivity using a tandem mass spectrometric technique and a cleanup procedure involving the use of an reversed phase solid phase extraction cartridge.

5. Conclusion

The mustard gas (HD) hydrolysis product, thiodiglycol (TDG), could be determined by a rather simple and rapid procedure using GC–MS after TBDMS derivatization from the aqueous, serum and urine samples. The inclusion of KCl in the aqueous sample during the evaporation step improved the derivatization efficiency of TDG, and a reasonable LOD value (5.4 ng/ml) was achieved. The LOD for a serum sample was determined to be 7.0 ng/ml, which satisfied the detection sensitivity for verifying HD exposure. In contrast, the LOD for the urine sample (110 ng/ml) was not sufficient to measure the background TDG level, and analytical improvements such as tandem MS and a cleanup pretreatment will be required.

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