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Short communication

Determination of metaldehyde in human serum by headspace solid-phase microextraction and gas chromatography–mass spectrometry

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ABSTRACT

A rapid headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS–SPME–GC–MS) method has been developed for the determination of metaldehyde in human serum samples. Metaldehyde is extensively used as a molluscicide for the control of slugs and snails, and cases of metaldehyde poisoning have been reported. Metaldehyde was headspace-extracted on a polydimethylsiloxane (PDMS) fiber at 70 °C for 25 min, desorbed, and analyzed rapidly by GC–MS. The method was validated for limit of detection (LOD), linearity, precision, and recovery. Although the recovery of the sample was very low, the method itself was rapid with a low detection limit of 0.25 μg/ml, R.S.D. value 12.6%, and linearity range 0.5–25.0 μg/ml (r^2 = 0.999). The results demonstrated that the SPME–GC–MS method for the analysis of metaldehyde is simple, rapid, solvent-free, and does not require any pre-analysis conversions.

1. Introduction

Metaldehyde is a cyclic tetramer of acetaldehyde (Fig. 1) and is extensively used as a molluscicide for the control of slugs and snails. Although it has low toxicity, cases of metaldehyde poisoning and death in animals and human beings due to accidental ingestion have been reported [1–4]. The symptoms of metaldehyde poisoning exhibited after oral intake include convulsions, abdominal pain, dizziness, nausea, irritation of oral mucosa, and seizures [1,3].

Analytical procedures for the determination of metaldehyde-employing techniques such as high performance-liquid chromatography (HPLC)-coupled fluorescence detection [5], gas chromatography (GC) detection after conversion of metaldehyde into acetaldehyde [6] or derivatization [7], and GC–mass spectrometric (GC–MS) detection [8] have been reported thus far. Although metaldehyde in plasma and urine has been analyzed directly using GC-flame ionization detection without any pre-analysis conversion [9], this method is not specific for metaldehyde. GC–MS is perhaps the most suitable method for metaldehyde analysis in plasma or urine. Although a GC–MS method for analysis of metaldehyde in stomach contents has been reported [8], a method for the determination of metaldehyde in biological fluids such as plasma or urine has not been reported yet.

The objective of the present study was to develop and validate a GC–MS method using the SPME technique for the determination of metaldehyde in human serum using 2,4-dimethylphenol as an internal standard (IS), and to use it for the evaluation of metaldehyde concentrations in the serum of patients with metaldehyde poisoning.

2. Experimental

2.1. Chemicals and standards

Metaldehyde was purchased from AccuStandard® (New Haven, CT, USA). 2,4-Dimethylphenol, was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Methanol was supplied by Merck (Darmstadt, Germany). Standard solutions of concentration 1.0 mg/ml were prepared in methanol and stored in amber glass-stoppered vials at −30 °C for up to 3 months. The standard solution of metaldehyde was diluted to 100 μg/ml and 10 μg/ml levels. SPME experiments were performed using a manual fiber holder supplied by Supelco (Bellefonte, PA, USA). Four commercially available fibers–PDMS, 100 μm; polyacrylate, 85 μm; PDMS/divinylbenzene (PDMS/DVB, 65 μm); and DVB/carboxen/PDMS (DVB/CAR/PDMS, 50/30 μm)–were purchased from Supelco. The serum sample for the validation study was collected from a volunteer.

Method validation was performed by evaluating the inter- and intra-assay precisions, recovery, and stability of low, medium, and high quality control (QC) concentrations. QC samples of 2 (low QC),

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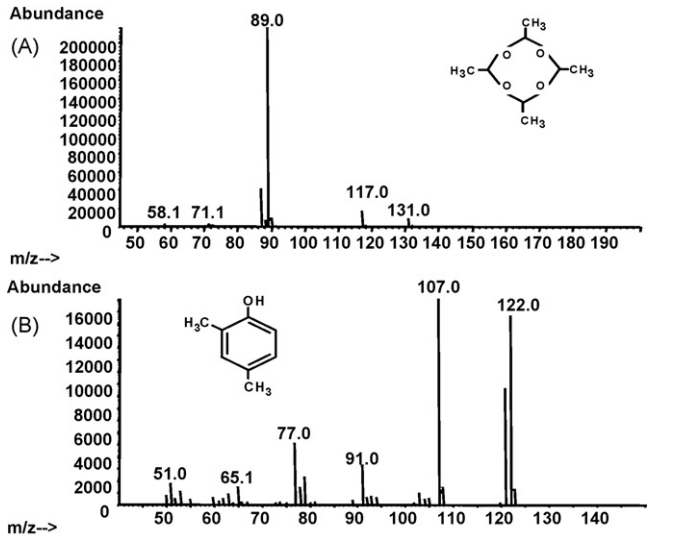

Fig. 1. GC–MS-EI spectra and chemical structures of metaldehyde (A) and 2,4-dimethylphenol (IS; B).

12 (medium QC), and 24 (high QC) μg/ml were prepared separately. All the solutions were stored at −30 °C and warmed to room temperature before use.

2.2. SPME procedure

The fibers were suitably conditioned in the GC injector port on first use and before each day's extraction in order to avoid any carry-over effect.

Analyses were performed using 4.0-ml vials sealed with hole-caps and silicone septa. The blank (plain serum, 0.2 ml) and the IS (1 mg/ml concentration, 5 μl) were added to the vials. The SPME fiber was introduced into the heated vial. After extraction, the fiber was retracted in a needle and introduced in the GC injector port; desorption was performed at 80 °C for 2 min. A fractional factorial experimental design was devised after selecting 4 suitable SPME fibers (PDMS, polyacrylate, PDMS/DVB, and DVB/CAR/PDMS). After evaluating each SPME fiber, parameters that had a significant influence on the extraction, such as time (5–30 min), temperature (60, 70, and 80 °C), and salting-out effect (0, 25, 50, 100, and 200 mg NaCl) were varied and the time required to attain equilibrium was evaluated. Blank serum samples spiked with metaldehyde were employed for this evaluation.

2.3. GC–MS

Since the report by Jones and Charlton [8] exclusively focused on metaldehyde analysis using GC–MS, the sequence of oven temperatures used in this study was standardized after suitable modifications.

GC–MS was carried out using an Agilent 6890N GC system (Agilent Technologies, Palo Alto, CA) equipped with an Agilent 5975B mass-selective detector. A crosslinked HP-5MS [(5%) phenyl-(95%) methylpolysiloxane] capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA) was used with helium (99.999% > grade purity) as the carrier gas. The sample was injected at an inlet temperature of 80 °C in the splitless mode. The mass spectra were obtained at 70 eV in the electron impact (EI) ionization mode. The source and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively. The flow-rate of helium was maintained at 1 ml/min using electronic pressure control. The sequence of temperatures in the chromatographic oven was as follows: 35 °C for 1 min followed by increments of 25 °C/min up to 280 °C at which point, the oven was allowed to remain at 280 °C for 1 min. The chromatographic run was completed in 11.8 min. A solvent delay of 5.0 min was incorporated to protect the filament from oxidation. Fig. 1 shows the EI mass fragmentation patterns of metaldehyde and the IS. The quantification of metaldehyde was performed in the selected-ion monitoring (SIM) mode, and the following ions were monitored: m/z 89.0 and 117.0 for metaldehyde and m/z 107.0 and 122.0 for the IS. The ions at m/z 89.0 and 107.0 were quantified with the retention times of metaldehyde and the IS set at 5.4 and 6.1 min, respectively.

2.4. Validation of the method

The linear range of the proposed method was studied by preparing a calibration curve for different concentrations of metaldehyde. The calibration curve was obtained for 5 calibration levels by adding 0.1 μg (0.5 μg/ml), 0.5 μg (2.5 μg/ml), 1 μg (5.0 μg/ml), 2.5 μg (12.5 μg/ml), and 5 μg (25.0 μg/ml) of calibration solutions to the HS vial. This was followed by addition of the IS and 0.2 ml blank serum to the HS vial. SPME and GC–MS were performed at optimum conditions after sealing the vial with a hole-cap and a silicone septum. The line of best fit for the relationship between the peak area ratio and the metaldehyde concentration was determined by linear regression.

The limit of detection (LOD) was determined using a low-concentration calibration solution (10 μg/ml). The LOD was defined as the concentration of metaldehyde that produced a chromatographic peak with a signal-to-noise ratio (S/N) greater than 3.

The precision of the method was expressed by the relative standard deviation (R.S.D.). To obtain the R.S.D. values, 6 replicate analyses of the blank with 3 QC concentrations were performed.

Recovery of the analyte was studied by adding the 3 QC standards to 0.2 ml of the blank. Three replicate analyses of each of the 3 QC samples were performed. The recovery was calculated by comparing the peak area of the extracted metaldehyde with the directly injected un-extracted component.

The effect of storage on the analysis in the course of a three-phase freeze–thaw cycle comprising storage at room temperature for 24 h, at 4 °C for 24 h, and storage in polypropylene tubes at −30 °C for 4 weeks was assessed by analyzing low, medium and high QC samples (n = 6).

2.5. Application

A 21-year-old healthy female was admitted to the emergency department at our hospital. She had attempted suicide by ingesting an unknown volume of molluscicide (3% metaldehyde and 3% Carbaryl) (Green Bait; Sankei Chemical Co., Ltd., Kagoshima, Japan). The insecticide, blood (without addition of any preservatives), urine, and gastric fluid were collected while the patient was in the resuscitation room. Emesis or other remarkable changes were not observed. Her skin and gastric mucous membrane were normal. Although cholinesterase activity was slightly reduced (154 U/l; normal, 180–430 U/l), the patient was conscious. She was released from our hospital 2 days later. The samples were stored at −30 °C until metaldehyde analysis was performed on them.

3. Results and discussion

3.1. Optimization of the SPME conditions

The efficiency of extraction using the SPME method is dependent on the fiber type, extraction time, and extraction temperature.

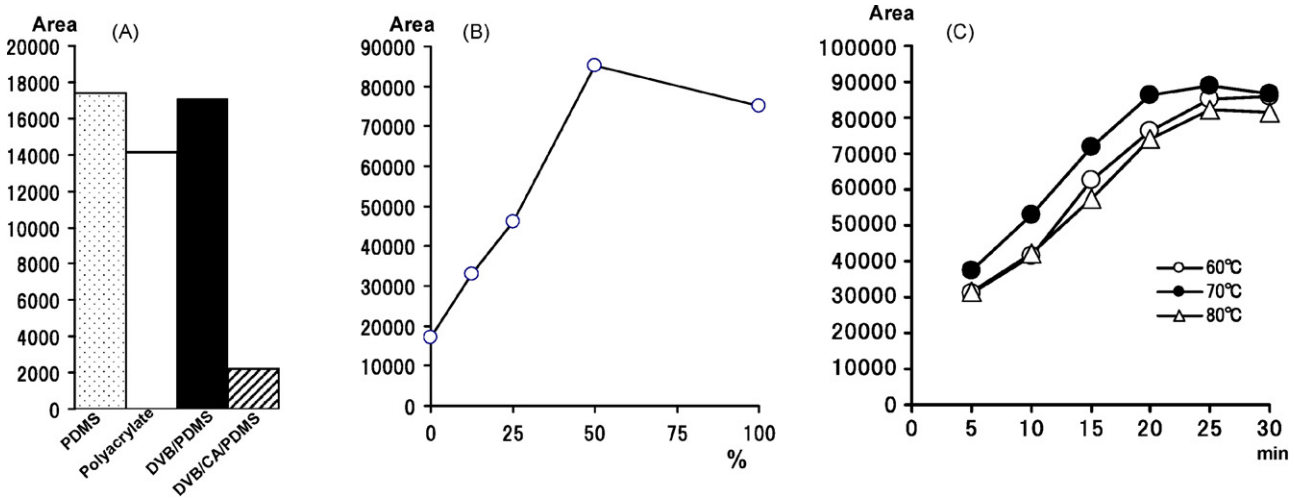

Fig. 2. (A) Average peak areas of spiked 0.2 ml serum samples obtained by HS–SPME with 4 different fibers; extraction temperature = 60 °C, extraction time = 20 min, desorption time = 2 min, $n = 3$. Metaldehyde concentration in each sample: 5 μg/ml; (B) effect of salting-out on the SPME procedure: effect of NaCl concentration assessed using PDMS fiber. Metaldehyde concentration in the sample: 5 μg/ml; (C) the effect of extraction temperature and time on the peak areas of metaldehyde assessed using PDMS fiber. Metaldehyde concentration in the sample: 5 μg/ml.

Our initial investigations were to determine the appropriate fiber. The affinity of the fiber for metaldehyde depends on the compounds coating it. We evaluated 4 commercially available fibers to determine which fiber was most effective in extracting metaldehyde.

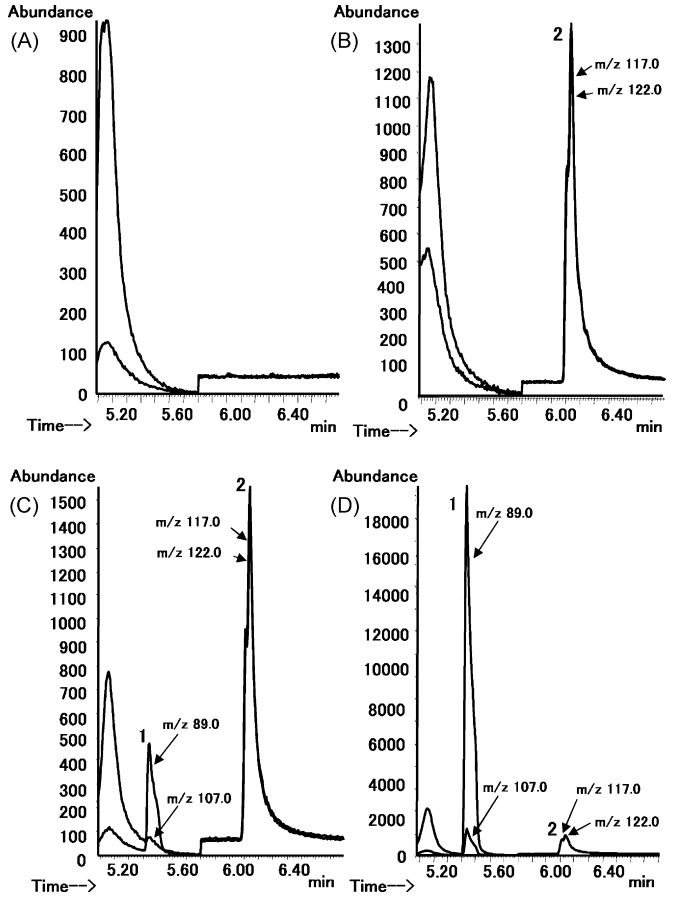

Fig. 3. GC–MS–SIM chromatograms obtained by SPME of (A) blank (plain human serum), (B) human serum with the IS (5 μg), (C) human serum with low QC (metaldehyde; 3 μg/ml) and the IS, and (D) serum of patient with metaldehyde poisoning with the IS. 1: metaldehyde, and 2: IS. Extraction temperature: 70 °C; time, 25 min; fiber, PDMS; desorption, 80 °C for 2 min.

Fig. 2A shows the effect of fiber coating on metaldehyde adsorption at 60 °C for 20 min. The extraction efficiency was marginally better in the case of the PDMS fiber than in the case of the other fibers under identical extraction conditions. While DVB/PDMS could not extract the IS, extraction using Carbowax produced some baseline noise around the IS peak. Therefore, the PDMS fiber was selected for further studies.

We also investigated the influence of the salting-out effect on the amount of metaldehyde extracted. Extraction recovery increased on the addition of salt to sample matrices as salt effectively reduced the solubility of metaldehyde. The effect of addition of NaCl to the samples is shown in Fig. 2B. The results show that the amount of metaldehyde extracted increased with the concentration of NaCl. The detected-metaldehyde area without NaCl was compared with the same area with NaCl. Blank serum was added with 25, 50, 100, and 200 mg NaCl and the mean metaldehyde area increased 2.5, 3.5, 6.5, and 5.7 times, respectively. The maximum amount of metaldehyde was extracted on addition of 100 mg of NaCl and decreased slightly with higher NaCl concentrations. Therefore, 100 mg of sodium chloride was used in the subsequent experiments.

Extraction temperature and time are important factors influencing metaldehyde adsorption on the SPME fiber. The effect of the extraction temperature and time on the amount of metaldehyde is shown in Fig. 2C. Although metaldehyde adsorption gradually increased from 5 to 25 min, it did not change significantly thereafter. Therefore, the extraction time of 25 min at 70 °C was used for further studies.

Jones and Charlton [8] reported that sensitivity increased when the injector temperature was increased to >80 °C. However, the linear range was reduced. Thus, we maintained the injector temperature at 80 °C for 2 min. Although this was a rather low temperature and short time for desorption, we confirmed that metaldehyde was completely desorbed from the fiber. Therefore, the fiber was not treated at high temperature for regeneration after desorption.

3.2. Validation of the method

The SPME–GC–MS chromatograms are shown in Fig. 3. The validation results are shown in Table 1. Under the optimal experimental conditions (i.e., fiber: PDMS; NaCl, 100 mg; extraction temperature:

Table 1
Inter- and intra-day precision of metaldehyde determination in human serum.

	Low QC (2 μg/ml)	Med QC (12 μg/ml)	High QC (24 μg/ml)
Mean (μg/ml)	1.9	12.1	24.1
S.D.	0.2	0.9	1.1
Inter-day R.S.D. (%)	12.6	7.1	4.4
Intra-day R.S.D. (%)	8.1	7.9	4.5
Recovery (%)	0.01	0.06	0.13

Table 2
Stability of metaldehyde.

Added	Found (μg/ml) (mean ± S.D.)	R.S.D. (%)
Three freeze and thaw cycles		
Low QC (2 μg/ml)	1.2 ± 0.2	14.3
Medium QC (12 μg/ml)	12.5 ± 0.9	7.2
High QC (24 μg/ml)	15.2 ± 0.7	2.9
4 weeks at −30 °C		
Low QC (2 μg/ml)	1.4 ± 0.1	7.5
Medium QC (12 μg/ml)	12.7 ± 0.8	6.3
High QC (24 μg/ml)	25.8 ± 0.4	1.6
24 h at 4 °C		
Low QC (2 μg/ml)	1.6 ± 0.1	4.8
Medium QC (12 μg/ml)	13.3 ± 1.0	7.1
High QC (24 μg/ml)	25.1 ± 0.6	2.4
Room temp. for 24 h		
Low QC (2 μg/ml)	1.2 ± 0.2	12.4
Medium QC (12 μg/ml)	11.7 ± 0.8	6.8
High QC (24 μg/ml)	23.8 ± 1.5	6.3

$n = 3$ each.

70 °C; extraction time: 25 min), precision, LOD, recovery, and the linear range of the proposed SPME method were studied.

The LOD was defined as the concentration of metaldehyde that produces a chromatographic peak with an S/N greater than 3. Analysis of a standard solution with low concentration (10 μg/ml) revealed a low LOD value (0.25 μg/ml) for the method.

The linearity was obtained by plotting the calibration curve of the peak area ratios versus the concentration of metaldehyde. The concentration range within which the method was linear was determined to be 0.5–25 μg/ml. The regression equation for metaldehyde was $y = 1.2197 \times -1.1775$. The coefficient of determination (r^2) was greater than 0.999. The average regression was always greater than 0.998.

Six replicate measurements of the 3 QC samples were used to calculate the R.S.D. value. The R.S.D. value of metaldehyde was between 4.4% and 12.6%.

The calculated recovery values for metaldehyde were between 0.01% and 0.13%. Usually, the recovery in SPME is lower (0.05–10%) than in other extraction methods such as liquid–liquid extraction [10]. In this study, the recovery of metaldehyde was less in the low-QC sample. The most probable reason would be that the vapor pressure of metaldehyde above the liquid phase was not proportional to the metaldehyde concentration but increased unproportionately (e.g. due to saturation effects, solubilization by methanol from the internal standard, binding to matrix constituents at low concentrations, etc.).

The results of the stability test are shown in Table 2. Stability testing in the course of a three-phase freeze–thaw cycle comprising storage at −30 °C for 4 weeks, storage at room temperature for 24 h, and at 4 °C for 24 h gave results within acceptable limits (±15% of nominal concentrations) except in the case of the low-QC samples.

The optimum SPME conditions standardized in this study were then applied to analyze the metaldehyde-poisoned human serum. Fig. 3D shows SPME–GC–MS chromatogram of the sample obtained from the poisoning case. The metaldehyde concentration was calculated to be 21.0 μg/ml. Moody and Inglis [4] reported that the peak metaldehyde concentration detected in the coma patient's serum was 125 μg/ml. Keller et al. [11] reported that metaldehyde concentration at 16 h postingestion is 10 μg/ml, which is shown by coma patients and those with seizures. Although metaldehyde concentration in our case was higher than that in Keller's case, coma was not recognized in our case. Carbaryl was not detected in the serum using LC–MS analysis.

4. Conclusions

In this study, an analytical method based on HS–SPME combined with GC–MS analysis has been developed for the determination of metaldehyde in human serum. This method is rapid, simple, and can be used for the direct analysis of metaldehyde without any preconversion. Moreover, the method is reproducible and linear within a relatively wide concentration range. Therefore, SPME–GC–MS is a rapid, simple, and efficient method for the determination of metaldehyde in serum.

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