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Liquid chromatography-tandem mass spectrometry method for the determination of thiosulfate in human blood and urine as an indicator of hydrogen sulfide poisoning

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ABSTRACT

Being a stable metabolite of hydrogen sulfide, thiosulfate has been utilized as an index for hydrogen sulfide poisoning (HSP). Thiosulfate analysis is mainly performed using gas chromatography/mass spectrometry (GC–MS) due to its high sensitivity and specificity. The GC–MS analysis requires two-step derivatizations of thiosulfate, and the derivative is not stable in solution as it has a disulfide moiety. To resolve this stability issue, we developed a novel analytical method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for monitoring the pentafluorobenzyl derivative of thiosulfate (the first reaction product of the GC–MS method) in this study. The established method exhibited high reproducibility despite being a more simplified and rapid procedure compare to the GC–MS method. Phenyl 4-hydroxybenzoate was used as an internal standard because 1,3,5-tribromobenzene which had been used in the GC–MS method was not suitable compound for LC-MS/MS with Electrospray ionization (ESI) negative detection. The linear regression of the peak area ratios versus concentrations was fitted over the concentration ranges of 0.5–250 μM and 0.25–250 μM in blood and urine, respectively. The validation results satisfied the acceptance criteria for intra- and inter-day accuracy and precision. Blood and urine samples from 12 suspected HSP cases were tested using this method. The thiosulfate concentration detected in the sample coincided well with that determined at the scene of each HSP accident.

1. Introduction

Hydrogen sulfide poisoning (HSP) occurs due to industrial poisoning incidents at chemical factories, human excreta, and sludge treatment plants as well as from exposure to volcano-released gases and oral ingestion of sulfur-containing pesticides or bath additives. In addition, recent HSP incidents involving hydrogen sulfide gas released from mixing sulfur-containing products with toilet cleaner have also been reported. Hitherto, confirmation of HSP has been performed by analysis of sulfides and their metabolites in body fluids and tissues of the deceased [1–5]. The results of sulfide analysis are drastically affected by the preservation temperature and time-length between death and completion of the autopsy as well as by sulfide production due to tissue decay [2]. However, thiosulfate (a metabolite of hydrogen sulfide) is contained in the blood and urine at low but consistent levels, and has been used as a reliable index for confirmation of sulfur poisoning [1,3–7].

Thiosulfate analysis is performed using high-performance liquid chromatography (HPLC) [8–12], gas chromatography (GC) [3,13], and GC/mass spectrometry (GC–MS) [4,5,7,13,14]. GC–MS, when used as an analytical method for determining the pentafluorobenzyl derivative of thiosulfates, has the advantage of providing conclusive identification of thiosulfates because it is highly sensitive and does not require cumbersome pretreatment procedures. The GC–MS method measures the disulfide compound (Scheme 1)

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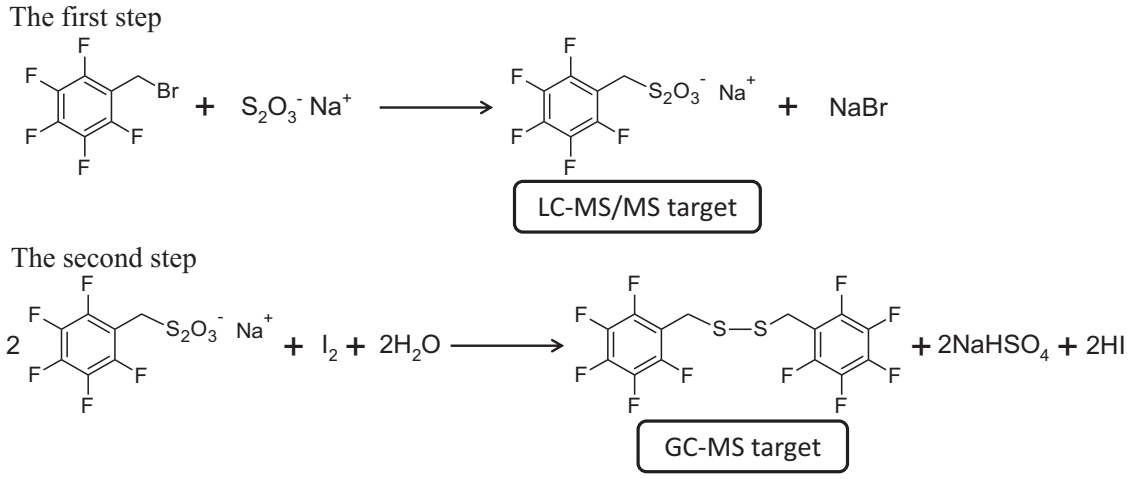

Scheme 1. Chemical derivatization reaction schemes for thiosulfate detection. GC–MS analysis requires two-step reactions to obtain the disulfide derivative. LC-MS/MS analysis is able to deal and detect a high polar pentafluorobenzyl derivative produced in the first step reaction.

derived from alkylation (step 1 reaction) of thiosulfate by pentafluorobenzyl bromide (PFB-Br) and following oxidation (step 2 reaction).

In recent qualitative and quantitative analyses of forensic samples, HPLC tandem mass spectrometry (LC-MS/MS) has been extensively employed. As long as the various analytical parameters of the target-compound are well defined, highly sensitive analysis with simple extraction of the target-compound(s) can be established. In this study, we performed our novel direct LC-MS/MS analysis of alkylated thiosulfate produced in the first step reaction of the GC–MS method.

2. Materials and methods

2.1. Chemical reagents

Aqueous solution, blood, and urine containing thiosulfate were prepared with 0.1 M sodium thiosulfate standard solution (Kanto Chemical Manufacturer, Tokyo). The internal standard material, phenyl 4-hydroxybenzoate (PHB, Tokyo Kasei Industrial Co. Ltd., Tokyo), was adjusted to 80 and 800 μM methanol. The derivative, pentafluorobenzyl bromide (PFB-Br; Tokyo Kasei Industrial Co. Ltd.), was treated with 5-μL type microcaps® (Drummond Scientific Co. Ltd. Broomall, PA). The following reagents (manufacturers) were purchased accordingly: L-ascorbic acid and methanol of special grades (Kanto Chemical Co. Ltd., Tokyo) as well as sodium chloride and formic acid of special grades (Wako Pure Chemicals Co. Ltd., Osaka) were used for reactions. Methanol for LC-MS (Kanto Chemicals Co. Ltd., Tokyo), millipore (Direct Q® 3UV)-filtered water, and ammonium formate (Sigma-Aldrich Co. Ltd., St. Louis, MO) were employed as the mobile phase in LC-MS/MS analysis. Millipore Ultrafree®-MC (pore size: 0.45 μm) was used for centrifugal filtration.

2.2. Biological samples

Forensic sample collection was performed as a routine part of our forensic work, following the autopsy guidelines and ethical guidelines of the Japanese Society of Legal Medicine. Blank blood and urine were donated from a healthy volunteer with informed consent and then stocked in a freezer until analysis. This study has been conducted with ethical approval by Asahikawa Medical University Research Ethics Committee (approval number: 16153).

2.3. LC-MS/MS analysis

HPLC (Prominences LC-20A System) and MS/MS (API 3200 Q-Trap System) were employed for thiosulfate determination. The measurement conditions for the LC-MS/MS analysis are indicated in Table 1.

2.4. Sample preparation

2.4.1. Qualitative examination

Ascorbic acid (50 μL, 200 mM), sodium chloride solution (50 μL, 5%), and 0.8 mL of methanol were first added to the blood (or urine) sample (0.1 mL) in a microtube before dripping a drop of PFB-Br. The mixture was vortexed vigorously for 1 min, subjected to 5-min sonication before centrifugation. The supernatant was used as the test sample for the qualitative analysis.

2.4.2. Quantitative analysis (standard addition method)

Ascorbic acid (50 μL, 200 mM), sodium chloride solution (50 μL, 5%), PHB solution (50 μL, 80 μM) and 0.8 mL of methanol were added to the blood (or urine) sample (0.1 mL) in each of two microtubes A and B. Microtube A was treated with distilled water (0.1 mL) while microtube B was treated with 0.1 mL of thiosulfate standard solution (0.1 μmol/mL) before dripping 1 drop of PFB-Br to each of the microtubes and 1-min stirring, followed by 5-min sonication and centrifugation. The supernatant (0.1 mL) of each microtube was transferred to a plastic test tube and diluted with about 10 mL of distilled water. A portion of each filtrate from A and B was thereafter analyzed.

The thiosulfate concentration in the sample was determined by the peak area ratio between PFB-S_2O_3H obtained from MRM chromatography and that of the internal standard ([A]: A, [B]: B) using the following equation:

$$\text{Thiosulfate concentration } (\mu\text{mol/mL}) = \mathrm{A}/(\mathrm{B}-\mathrm{A}) \times \text{added thiosulfate}(\mu\text{mol})\}/\text{sample volume}(\text{mL})\}$$

2.5. Validation procedures

The established method was validated according to the US Food and Drug administration (FDA) guidelines [15]. The selectivity, accuracy, precision, calibration curve, sensitivity, reproducibility and stability were evaluated. Moreover, the amount of authentic