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Short communication

Analysis of acetylene in blood and urine using cryogenic gas chromatography–mass spectrometry☆

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ABSTRACT

A method for quantitative analysis of acetylene in blood and urine samples was investigated. Using cryogenic gas chromatography–mass spectrometry (GC–MS), acetylene was measured with isobutane as the internal standard in the headspace method, which revealed a linear response over the entire composite range with an excellent correlation coefficient, both in blood (R = 0.9968, range = 5.39–43.1 μg/ml) and urine (R = 0.9972, range = 2.16–10.8 μg/ml). The coefficients of variation (CV) for blood ranged from 2.62 to 11.6% for intra-day and 4.55 to 10.4% for inter-day. The CV for urine ranged from 2.38 to 3.10% for intra-day and 4.83 to 11.0% for inter-day. The recovery rate as an index of accuracy ranged from 83 to 111%. The present method showed good reliability, and is also simple and rapid. In actual samples from a charred cadaver due to acetylene explosion, the measured concentrations of acetylene by this method were 21.5 μg/ml for femoral vein blood, 17.9 μg/ml for right atrial blood, 25.5 μg/ml for left atrial blood and 7.49 μg/ml for urine. Quantification of acetylene provides important information, because the acetylene concentration is a vital reaction or sign. For example, when acetylene is filled in a closed space and then explodes, in antemortem explosion, the blood acetylene concentration of the cadaver might be significant. On the other hand, in postmortem explosion, acetylene is not detected in blood. Furthermore, when several victims are involved in one explosion, comparison of the sample concentrations can also provide useful information to establish the conditions at the accident scene; therefore, the present method is useful in forensics.

1. Introduction

Acetylene is commonly used for welding in small-scale industries due to the high temperature of the flame; however, it is extremely flammable, and mixtures with air containing between 2.5 and 82% acetylene are explosive on ignition [1,2]. Accidental or suicidal cases of death due to inhalation of acetylene and/or involving trauma induced by acetylene explosion are occasionally encountered in forensic science practice [1,3,4]. The risk of acetylene inhalation or acetylene explosion for industrial workers is also a problem from a health point of view [3–7].

In forensic science practice, detecting the causative agent of an explosion in body fluid is very important when victims are found at an explosion scene. We experienced an actual acetylene explosion case, and it was necessary to analyze acetylene in blood and urine immediately. Previously, the detection of acetylene in the lungs by gas chromatography–mass spectrometry (GC–MS) with the headspace method was reported as a case report [4]; however, in that report, Williams et al. [4] considered that the analytical method was unreliable for quantitative analysis from blood. Few reliable methods have been reported to quantify acetylene in biological samples. We therefore investigated rapid and reliable methods for measuring acetylene in blood and urine.

Analysis using a cryogenic oven with GC is known as a sensitive technique to detect volatile organic compounds in biological samples such as blood and urine [8,9]. To our knowledge, the first report dealing with GC with a cryogenic oven temperature device in biological samples was the analysis of chloroform or methylene chloride in whole blood [10]. This device was originally designed to rapidly cool the oven to reduce analysis time. Watanabe et al. [10] reported that the method using that device was very sensitive and showed low background noise. Accordingly, we used this assay to analyze acetylene. The molecular weight of acetylene is 26.04, which is less than that of nitrogen. Good chromatographic

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separation from air and low background noise are indispensable to quantify acetylene.

In this paper, we describe a rapid and reliable method for measuring acetylene using cryogenic gas chromatography–mass spectrometry (GC–MS). Further, the method developed in this study was applied to measure acetylene in blood and urine from our actual autopsy case.

2. Experimental procedures

2.1. Materials

2.1.1. Chemicals

Acetylene (>98%) and isobutane (>99.9%) were obtained from Takachiho Chemical Industrial Co., Ltd. (Tokyo, Japan). Acetylene-d_2 (>99%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

2.1.2. Tools

The gases were measured using gastight syringes (Hamilton Co., Reno, NV, USA). The 10-ml glass vial used for headspace sampling could be sealed with a polytetrafluoroethylene (PTFE)-coated silicon septum and a screw cap. The vials were evacuated with nitrogen gas before use.

2.1.3. Specimens for calibration curves

Acetylene-negative (blank) blood and urine specimens for calibration curves were obtained from healthy volunteers with their consent.

2.1.4. Specimen from an actual autopsy case

A male cadaver in his thirties was found in an exploded truck cabin. According to the forensic autopsy findings and police information, the victim was suspected to have died in an acetylene explosion. His blood and urine were collected and stored at −20 °C prior to analysis.

2.1.5. Sample preparation

Acetylene and isobutane were collected into separate plastic bags with silicone caps by water substitution from cylinders.

In a glass vial, 200 μl blood or 1000 μl urine was added to 4 μl isobutane as an internal standard (I.S.). The mixture was kept at room temperature for 10 min. Headspace (200 μl) was injected into the GC–MS instrument.

2.2. Gas chromatography–mass spectrometry

GC–MS analyses were performed on a QP-2010Plus equipped with a cryogenic oven temperature device in positive electron impact (EI) mode (Shimadzu Co., Kyoto, Japan). An electrically operated valve introduces liquid carbon dioxide at a rate appropriate to cool the oven to the desired temperature. GC–MS conditions were as follows: Rtx®-1 capillary column, 60 m × 0.32 mm I.D., 3.0 μm film thickness (Restek, Bellefonte, PA, USA); injection port temperature 160 °C; injection mode, split injection (split ratio 18:1); column oven temperature, initially held at −10 °C for 3 min, then increased at 50 °C/min to 90 °C, and held at 90 °C for 2 min; carrier gas, helium; linear velocity, 39.8 cm/s (constant); ion source temperature, 200 °C; interface temperature, 160 °C; ionization energy, 70 eV; scan range, *m*/*z* 20–200; scan cycle, 0.06 s. All data were collected in full-scan mode.

2.3. Quantitative analysis

The amount of acetylene in a sample was estimated using a mass chromatogram reconstructed with a molecular ion at *m*/*z* 26 for acetylene and a fragment ion at *m*/*z* 43 for isobutane.

Calibration curves were prepared using the peak area ratios of acetylene to isobutane against acetylene amounts added to blank blood (1.08–8.63 μg per 0.2 ml) and blank urine (2.16–10.8 μg per 1.0 ml), by plotting five concentrations for each. Then, 9.64 μg of isobutane was added to each sample, and calculated using the ideal gas law under the atmospheric pressure 1010 hPa and temperature 20 °C. To prevent acetylene from being volatilized, we divided the samples into two bottles for storage. One was used to establish measurement conditions, and the other for measuring concentration. We decided the calibration concentrations from the results of sample injections from one group of bottles.

3. Results and discussion

3.1. Method evaluation

Method validation parameters are discussed. Definitions and criteria were adopted according to the analysis reported by Rozet et al. [11].

3.1.1. Selectivity

Generally, the stable isotope-labeled analogue is a desirable internal standard for quantitative analysis in GC–MS. We compared isobutane with acetylene-d_2; however, because acetylene-d_2 possesses fragment ion *m*/*z* 26, *ca.* 30% of the molecular ion (Fig. 1), it was considered that acetylene-d_2 is not suitable under these conditions; therefore, we used isobutane as the I.S.

Under GC–MS conditions, acetylene and isobutane appeared at 2.91 min and 5.41 min, respectively. The characteristic mass spectrum of acetylene indicating the molecular ion at *m*/*z* 26 was also observed. The acetylene peak was clearly distinguishable from air peaks (containing oxygen, nitrogen, argon and carbon dioxide) and I.S. Acetylene and isobutane used as standard substances were not contaminated in this analysis.

3.1.2. Linearity

Calibration curves were linear within the range of 1.08–8.63 μg per 0.2 ml blood, $y = 0.0321x - 0.0264$ ($R = 0.9968$); 2.16–10.8 μg per 1 ml urine, $y = 0.1412x - 0.0338$ ($R = 0.9972$).

To confirm linearity in a wider range, samples were calibrated at eight different concentrations for each matrix (0.532–53.2 μg per 0.2 ml blood, 0.532–53.2 μg per 1.0 ml urine) together with a fixed amount of I.S. (9.51 μg calculated under 1013 hPa atmospheric pres-

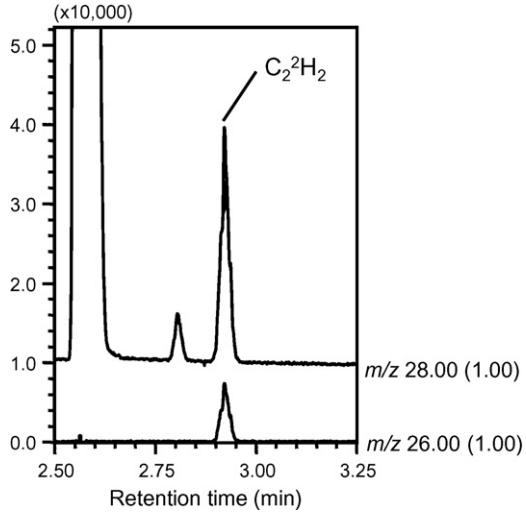

Fig. 1. Mass chromatograms reconstructed with the molecular ion for acetylene-d_2 (*m*/*z* 28) and without deuterium (*m*/*z* 26). Sample: headspace of blank blood with added acetylene-d_2.

Table 1
Intra-day and inter-day precisions expressed by CV (%) ($n = 5$).

Intra-day (%)			Inter-day (%)		
5.32 μg/ml	10.6 μg/ml	42.6 μg/ml	5.32 μg/ml	10.6 μg/ml	42.6 μg/ml
Blood					
11.6	2.62	3.24	10.4	6.89	4.55
Intra-day (%)			Inter-day (%)		
2.13 μg/ml	4.26 μg/ml	10.6 μg/ml	2.13 μg/ml	4.26 μg/ml	10.6 μg/ml
Urine					
3.10	2.92	2.38	4.83	11.0	5.00

sure and 25 °C). The correlation coefficients were 0.9984 for blood and 0.9973 for urine. The low limit of detection (signal-to-noise ratio = 3) of acetylene was estimated to be *ca.* 0.53 μg/ml (blood) and 0.11 μg/ml (urine). The present method does not inquire much higher sensitivity.

3.1.3. Precision

Intra-day and inter-day precisions were established for three different concentrations (Table 1). The coefficients of variation (CV) for blood ranged from 2.62 to 11.6% for intra-day and 4.55 to 10.4% for inter-day. The CV for urine ranged from 2.38 to 3.10% for intra-day and 4.83 to 11.0% for inter-day.

3.1.4. Accuracy

Accuracy was evaluated by comparing the results of the calibration curve for each matrix and by adding known amounts of acetylene to samples. The results are illustrated in Table 2. Recovery was calculated as the ratio percent between expected and measured concentrations [12]. Feinberg [13] reported that the acceptable limit of recovery was 75–125%; therefore, the results

Table 2
Accuracy was evaluated by comparing the results of the calibration curve for each matrix and by adding known amounts of acetylene to samples.

Expected (μg/ml)	Measured ($n = 5$) (mean ± S.D.) (μg/ml)	Recovery[a] (%)
Blood		
10.8	11.1 ± 0.4	103
42.6	41.3 ± 1	97
Urine		
2.13	2.36 ± 0.04	111
10.6	8.76 ± 0.2	83

[a] Recovery is calculated as the ratio percent between expected and measured concentration.

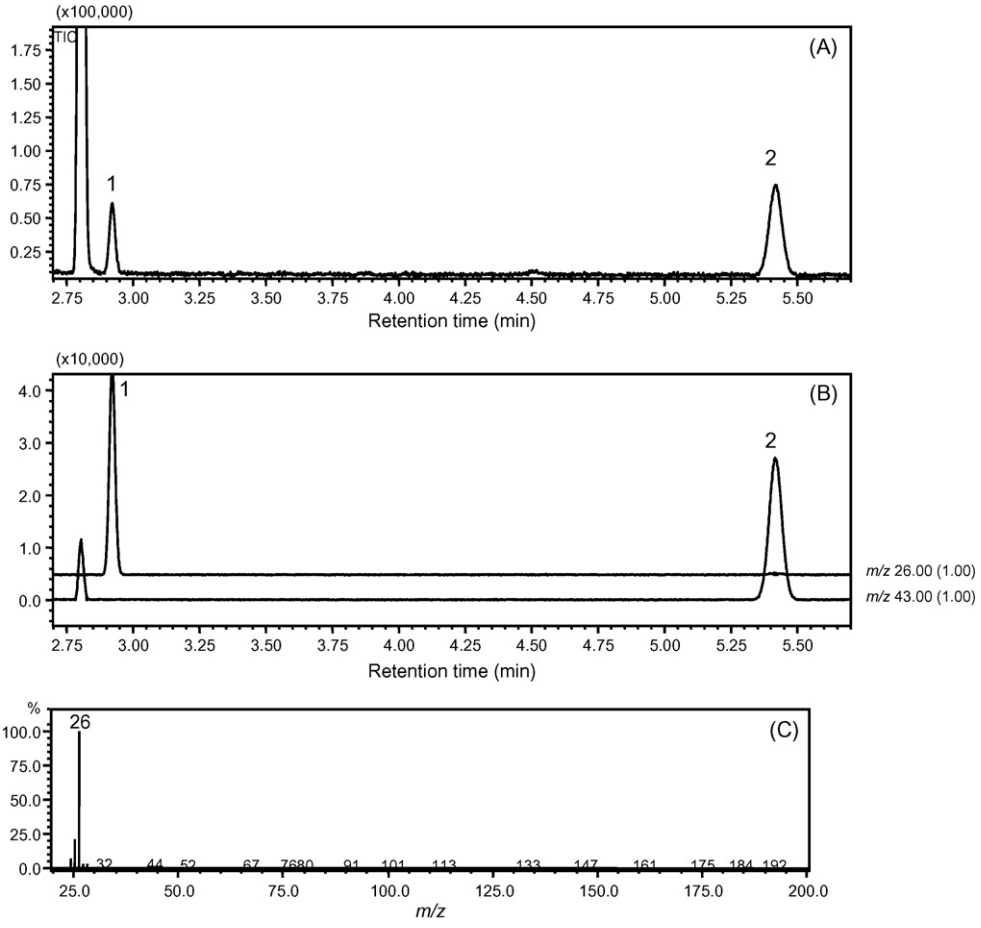

Fig. 2. Detection of acetylene in the victim's peripheral blood sample. (A) Total ion chromatogram; (B) mass chromatograms reconstructed with the molecular ion for acetylene and the fragment ion for I.S.; (C) mass spectrum identified with acetylene. (1) Acetylene; (2) isobutane (I.S.).

showed that accuracy was satisfactory in the range 10.8–42.6 μg/ml for blood and 2.13–10.6 μg/ml for urine.

3.2. Application on biological samples from an actual case

The total ion chromatogram (TIC), mass chromatogram and mass spectrum obtained from the blood of the victim found at the scene of an acetylene explosion are shown in Fig. 2. We analyzed the samples 2 months after collection. The concentrations of acetylene were estimated as 21.5 μg/ml for femoral vein blood, 17.9 μg/ml for right atrial blood, 25.5 μg/ml for left atrial blood, and 7.49 μg/ml for urine. Acetylene is highly volatile and postmortem measurements might not represent the concentration of gas at the time of death; the measured concentrations of acetylene in blood and urine are considered to be lower than the accident scene concentrations.

4. Conclusions

Quantitative analysis of acetylene was made possible in blood and urine samples by the present method. This method showed good reliability, and is also simple and rapid. In acetylene explosion cases, the quantification of acetylene in blood and/or urine from cadavers gives important information, because the acetylene concentration is a vital reaction or sign. For example, when acetylene is filled in a closed space and then explodes, in antemortem explosion, the blood acetylene concentration of the cadaver might be significant. On the other hand, in postmortem explosion, acetylene is not detected in blood. Furthermore, when several victims are involved in one explosion, comparison of the sample concentrations can also provide useful information to establish the conditions at the accident scene.

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