Quantitative determination of H₂ in human blood by ²²Ne-aided gas chromatography–mass spectrometry using a single quadrupole instrument

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Abstract

Here, we present a quantitative method for H₂ detection by gas chromatography–selected ion monitoring-mass spectrometry (GC–SIM-MS) using a single quadrupole instrument. Additionally, the developed method was applied in the detection of H₂ in human blood by GC–SIM-MS analysis using the existing ²²Ne in the air as an internal standard (IS). H₂ was analyzed by GC–SIM-MS using a single quadrupole instrument with double TC-Molsieve 5A capillary columns for the separation of permanent gases. The detections of H₂ (analyte) and ²²Ne (IS) were performed at m/z 2 and 22, respectively, by GC–SIM-MS. The analyte and IS were separated using He as the carrier gas. The ratio of the peak area of H₂ to ²²Ne was employed to obtain the calibration curve for H₂ determination in the gas phase. The proposed GC–SIM-MS method exhibited high sensitivity in terms of the limits of detection (LOD) (1.7 ppm) and quantitation (LOQ) (5.8 ppm) for the H₂ analysis. The developed quantitative assay of H₂ in the headspace of blood samples achieved high repeatability with a relative standard deviation (RSD) of 1.4–4.7%. We successfully detected and quantified H₂ in the headspaces of vacuum blood-collection tubes containing whole blood from 11 deceased individuals with unknown causes of death employing the developed GC–SIM-MS method. The quantitative value of H₂ ranged from 5 to 905 ppm. The proposed GC–SIM-MS method was applicable to the quantitative assay of H₂ in biological samples without tedious pretreatment requirements.

Keywords: H₂ analysis, ²²Ne, GC–SIM-MS, quadrupole, SIM
Introduction

H₂, an inorganic gas, is colorless, odorless, extremely flammable, and present in the atmosphere at a concentration of 0.5 ppm.¹ It is well-known that H₂ is generated by the putrefaction of living tissues, and that it is typically employed as an indicator of putrefaction.²⁻⁵ Bajanowski et al. analyzed the gas in the heart of a deceased (detecting 0.5–50% of H₂) to determine if the gas was produced by air embolism or putrefaction.²,³ Further, to investigate the causes of death and poisoning, the toxicological analysis of the human blood is extremely important. Ethanol in the blood may be produced postmortem due to putrefaction,⁶ and it is extremely difficult to distinguish whether it is from antemortem ingestion or a postmortem product. Although it has been reported that 1-propanol was applied as an indicator of putrefaction,⁷,⁸ its production mechanism is not uniform⁵ and 1-propanol may not always be detectable.⁹ Therefore, more indicators are required to identify putrefaction, and H₂ may be employed as one. However, H₂ analysis in human blood is rarely reported in forensic science and in the medical field, with the exception of the paper by Ono et al.¹⁰ This is because H₂ possesses an extremely low solubility in blood and because H₂ diffuses during pretreatment.

Ono et al. performed H₂ aspiration on patients with ischemic brain disease and analyzed the H₂ concentration in the headspace of the blood samples by gas chromatography (GC) using a gas sensor. Nevertheless, there was a problem with the pretreatment method, and accurate quantitation was not performed. As stated above, there have been no reports on the accurate and quantitative analysis of H₂ in blood.

The determination of H₂ in forensic science is typically by GC with a thermal conductivity detector (GC-TCD).²⁻⁴,¹¹⁻¹³ H₂, detected by the lactose breath test, is employed as a biomarker of lactose intolerance and features GC-TCD¹⁴ and GC with a metal oxide detector.¹⁵,¹⁶ The highly sensitive
discharge ionization detector (DID), He ionization detector (HID), and dielectric-barrier discharge ionization detector (BID) can also be used to detect H₂. Previous reports have indicated that the detection of H₂, possessing a lower molecular weight than He (employed as the carrier gas), was impossible by GC coupled with mass spectrometry (GC–MS) using a single quadrupole instrument. Therefore, Varlet et al. indirectly detected H₂ as protonated He by GC–MS using a special column, which consisted of two capillary columns set in parallel: molecular sieve capillary and porous-layer open tabular (PLOT) capillary columns, designed for gas analysis. Conversely, in environmental and earth sciences, the analysis of H₂ is typically performed on an isotope-ratio mass spectrometer (IRMS) and quadrupole ion trap mass spectrometer (QITMS). However, these instruments present challenges, including specialty, high cost, low adaptability, and narrow dynamic range.

Here, we present an H₂ analysis method employing GC–MS with a single quadrupole instrument that affords high versatility. Further, the application of the ²²Ne-aided GC coupled with selected ion monitoring MS (GC–SIM-MS) method, which was developed in our previous report for the quantitative determination of H₂ in the headspace of blood is described.

Materials and methods

Materials

H₂ (99.99999% purity, specification value) was supplied by an H₂ generator NMH-100 (maintenance-free apparatus, Air Tech, Yokohama, Japan). He (purity > 99.995%) was purchased from Taiyo Nissan (Tokyo, Japan). H₂ of 1% concentration in He was prepared by diluting pure H₂ with He in an Al bag (1 L, GL Sciences, Tokyo, Japan) using gas-tight syringes (SGE Analytical Science, Melbourne, Australia). H₂ concentrations of 5 ppm–10% in the air were prepared by diluting pure H₂ with
air in the Al bag using the gas-tight syringes. The sample gas was injected into the GC–MS system using
the gas-tight syringe. A lock-type needle (inner diameter, 0.3 mm; length, 40 mm, GL Sciences) was
utilized with the syringe described above. Commercially available human whole blood (from a healthy
adult) was purchased from Cosmo Bio Co. (No.12081545, Tokyo, Japan).

GC–MS analysis for the detection of the H₂

The GC–MS settings for the detection of H₂ were modified from those described in our previous
report. The GC–MS analysis was performed using a Shimadzu GC–MS QP2010Plus (Kyoto, Japan). A
TC-Molsieve 5A capillary column (30 m × 0.32 mm i.d., film thickness 30 µm, GL Sciences) was
employed for the gas separation. In this study, either a single or double column (two-connected
TC-Molsieve 5A capillary columns with lengths of 60 m; Fig. 3) capillary was adopted for the GC–MS
analysis. In the single-column assay, a particle trap (2.5 m × 0.32 mm, GL Sciences), which prevents the
column filler from contaminating the mass detector, was connected to the analytical column on the side of
the detector. In the double-column assay, two TC-Molsieve 5A capillary columns were connected in
series and the particle trap was connected to the analytical double column on side of the detector. The GC
conditions were as follows, except in the injection mode: carrier gas, He; column temperature, 35 °C;
purge flow rate, 3.0 mL/min; and injection temperature, 40 °C. The MS conditions were as follows:
electron ionization (EI) mode; ionization voltage, 70 eV; emission current, 150 µA; scan range, m/z 2–50
in the full scan mode for H₂; monitoring ions, m/z 2 and 22 in the SIM of H₂ and ²²Ne, respectively. Split,
splitless, and direct-injection modes were adopted as the sample injection methods, and the best condition
was the split (5:1) as will be described later.

Calibration and repeatability
For the quantitation of H₂, a calibration curve was established by the GC–SIM-MS analysis using atmospheric ²²Ne (1.68 ppm) as the IS. The H₂ standard gas diluted with air to concentrations of 5–to 1,000 ppm was prepared, and the IS was added automatically by dilution with air. The detections of H₂ and ²²Ne were conducted in SIM mode (H₂: m/z 2 and ²²Ne: m/z 22). The limits of detection (LOD) and limits of quantification (LOQ) were calculated at S/N ≥ 3 and ≥ 10, respectively. Repeatability, in terms of the relative standard deviation (RSD) and trueness (%), was determined by analyzing the 100 ppm H₂ standard gas samples (n = 4).

Quantitative ²²Ne-aided GC–SIM-MS analysis of H₂ in whole blood specimens

To apply the quantitative ²²Ne-aided GC–SIM-MS analysis to H₂ detection in blood, the whole blood sample from the heart was transferred to a 10 mL vacuum blood-collection tube containing sodium heparin (Terumo Co., Tokyo, Japan) and refrigerated at 4 °C until the analysis. Prior to the analysis, the blood tube was allowed to stand for 1 h at 25 °C. After achieving equilibrium in the headspace phase at 25 °C, 1.0 mL of the headspace was injected into the GC–SIM-MS system using the gas-tight syringe fitted with a push-pull valve (SGE Analytical Science). For the ²²Ne-aided GC–SIM-MS analysis as part of the calibration, H₂ gas samples diluted with air were employed. Since air contains a constant amount of ²²Ne (1.68 ppm), the air-dilution method determined the amount of ²²Ne as the IS that was spiked in the headspace. The commercially available blood sample was employed as a blank.

Method repeatability for whole blood specimens.

The commercially available human whole blood (2.0 mL) was injected into a vacuum blood-collection tube and 11 or 66 µL of H₂ standard gas (10% in air) was quickly spiked in the tube headspace by bubbling at 100 or 500 ppm using a gas-tight syringe fitted with a push-pull valve (50 or
100 µL volume). After standing at 25 °C for 1 h, 1.0 mL of the headspace of the vacuum blood-collection
tube was sampled using a gas-tight syringe with a push-pull valve and subjected to GC–SIM-MS analysis. The intraday \( n = 5 \) and interday \( n = 9 \) repeatabilities of the present method in terms of their RSDs (%) were investigated for the whole blood samples spiked with \( \text{H}_2 \). The interday repeatability was achieved for three consecutive days with triplicate determinations.

Ethical approval

This work did not involve any experiment with human participants or animals by any of the authors. The analysis of the toxic substance(s) in the whole blood samples collected from cadavers was requested by judicial authorities through official documentation.

Results and discussion

Detection of \( \text{H}_2 \) by GC–MS using a single quadrupole instrument

\( \text{H}_2 \), as had been previously reported, is difficult to detect by GC–MS using a single quadrupole instrument.\(^{11-13} \) The \( \text{H}_2 \) assay was performed in full scan mode using a TC-Molsieve 5A capillary column (single column, 30 m × 0.32 mm) for the inorganic gas analysis. The analysis of the \( \text{H}_2 \) standard gas (1% in He) revealed a peak at a retention time of 1.4 min (Fig 1a). A mass spectrum at \( m/z \ 2 \) as the base peak was obtained from this peak (Fig. 1b). In the total ion chromatogram (TIC), the peaks of Ar, O\(_2\) (1.9 min), and N\(_2\) (3.3 min) contaminating the needle of the syringe from the air were also detected. The contamination will not affect the quantitative analysis, because the concentration of hydrogen in the air (0.5 ppm) is extremely low and the volume of the needle (2.8 µL) is only about 0.3 % of the sample injection volume. Since it was possible to detect \( \text{H}_2 \) by MS using a single quadrupole instrument, we
attempted to analyze the H\textsubscript{2} standard gas (100 ppm in the air) by GC–SIM-MS. As shown in Fig. 2, the peak was detected at 1.4 min, which was the same retention time as obtained in the full scan mode, indicating that H\textsubscript{2} can be detected in SIM mode. However, tailing and deterioration of S/N occurred due to the proximity of the negative peaks of Ar and O\textsubscript{2}. Consequently, the LOD of H\textsubscript{2} deteriorated to 13.4 ppm.

**GC–SIM-MS analysis**

As described above, since tailing was observed when using a single column, we were concerned about the decrease in the detection sensitivity. Therefore, GC–SIM-MS analysis for H\textsubscript{2} detection using a double-column system was employed with reference to our previously developed method.\textsuperscript{18} The influences of the carrier gases (He, Ar, and N\textsubscript{2}) were examined (data not shown), and H\textsubscript{2} was detected only when He, which possesses the smallest ionization cross-section of all the gases tested (He: 0.365 × 10\textsuperscript{-20} m\textsuperscript{2}, H\textsubscript{2}: 1.10 × 10\textsuperscript{-20} m\textsuperscript{2}, Ar: 3.15 × 10\textsuperscript{-20} m\textsuperscript{2}, and N\textsubscript{2}: 2.65 × 10\textsuperscript{-20} m\textsuperscript{2}), was employed.\textsuperscript{21} The peak shape was also good, hence He was employed as the carrier gas. H\textsubscript{2} was deployed as the carrier gas for the He detection, and the gas samples were injected in split (2:1) mode.

The application of He, which is more viscous than H\textsubscript{2}, as the carrier gas was presumed to change the behaviors of separation and detection. Therefore, the injection method was first optimized (data not shown). H\textsubscript{2} was detected only when the split injection method (5:1) was employed, whereas no obvious peaks were observed in the spectra obtained using the other methods.

Furthermore, the optimum split ratio was examined between the range of 2:1–20:1 (data not shown). The peak of H\textsubscript{2} sharpened as the split ratio increased, but since the peak area decreased, a split ratio of 5:1 that achieved the largest S/N ratio was optimal. As shown in Fig. 4, the H\textsubscript{2} standard gas (100 ppm in the
air) was analyzed by GC–SIM-MS under the optimized conditions, and H₂ (3.3 min) and ²²Ne (concentration in the air: 1.68 ppm) were detected with good peak shapes.

Moreover, as shown in Fig. 4, extremely good linearity was obtained between the H₂ concentration (5–1000 ppm) and the peak area of H₂ with a correlation coefficient of 0.9994. Therefore, H₂ was quantitatively ionized in the mass spectrometer. On the other hand, a decrease in responsibility of mass spectrometer was observed at 10,000 ppm of hydrogen. This result strongly suggests that GC–SIM-MS, using a single quadrupole instrument, was effective for detecting H₂.

Method reliability

Extremely good linearity was obtained between the H₂ concentration (5–1000 ppm) and the peak ratio of H₂ to ²²Ne as an IS with a correlation coefficient of 0.9992 (Fig. S1 in Supporting Information). Subsequently, since the RSD and trueness of the peak ratio of H₂ (100 ppm) to IS were 4.5 and 97.6%, respectively, it was implied that high reproducibility was achieved. The LOD and LOQ, which were 1.7 and 5.8 ppm, respectively, implied that high detection sensitivity was achieved. In this study, the peak intensity of ²¹Ne (ex.: 3,983) was extremely low when H₂ standard gas (100 ppm in the air) was analyzed under the optimized conditions. Therefore, ²²Ne (peak area: 144,702) was assigned a detection level that was equivalent to the peak intensity obtained in the measurement range of H₂. Additionally, the reproducible peak detection of ²²Ne (peak area units: 145,074 ± 2,328; n = 8; RSD, 1.6%) supported the utilization of ²²Ne as an IS.

This determination method for H₂ was evaluated by examining its intraday and interday repeatabilities in whole blood samples spiked with H₂. As shown in Fig. 5 and Table 1, the RSDs of the intraday and interday repeatabilities at both H₂ concentrations were < 4.7%. Thus, the application of ²²Ne as an IS afforded satisfactory reproducibility for the quantitative analysis of H₂.
As in our previous report, according to Henry's law, the water solubilities of both $^{22}\text{Ne}$ and $\text{H}_2$ are low, therefore, it was not necessary to consider the difference in the partition ratio between the gas completely sealed in the vacuum blood-collection tube and the gas species. Therefore, in this study, it was speculated that the blood volume and the degree of vacuum of the blood-collection tube can be neglected during blood sample preparation and the $^{22}\text{Ne}$-aided GC–SIM-MS analysis.

On the other hand, as described above, the decrease in the response of the mass spectrometer of high concentration $\text{H}_2$ left a problem in the quantitative analysis for the gas phase from blood specimen.

**Method application**

The quantitative analysis of $\text{H}_2$ was performed employing the developed $^{22}\text{Ne}$-aided GC–SIM-MS method for the whole blood samples of 11 deceased individuals, whose causes of death were unknown, jumping, hanging and fire. 1-Propanol and ethanol in all blood samples were analyzed according to the general method by headspace gas chromatography. Each whole blood sample (ca. 4.8–10.5 mL) was obtained from the heart of each cadaver by puncturing from outside the body using a plastic syringe (10 mL, Nipro Co., Osaka, Japan) with a catheran needle (18G; length, 70 mm, Nipro Co.). The blank specimen was a commercially available human whole blood sample (no. 12081545). As shown in Fig. 6b (case 1: 187 ppm) and Table 2, the $\text{H}_2$ concentration in the headspace of the blood was determined to range from 5 to 905 ppm. Cases 4 and 7 were outside the calibration curve, hence the data were only for reference. Conversely, the $\text{H}_2$ peak was not observed in the blank blood (Fig. 6a). In previous medical studies, it was reported that an $\text{H}_2$ concentration of 0.5 $\mu\text{mol/L}$ (12.2 ppm) could be detected by aspirating a high concentration of $\text{H}_2$ (3–4%) into a patient while monitoring the blood $\text{H}_2$ concentration. In this study, it was possible to detect $\text{H}_2$ at the same level (case 2: 29 ppm, case 8: 5 ppm, case 9: 21 ppm) as that previously reported. Each case was inspected within 1 day after death and postmortem blood
collection. In cases 3, 4, and 7, the number of days after death was unknown, and the postmortem blood samples were quickly collected during an autopsy. High concentrations of \( \text{H}_2 \) were detected in the three cases (case 3: 905 ppm, case 4: 15,509 ppm, case 7: 10,768 ppm), and it was inferred from the blood properties (color, viscosity, and smell) and the conditions of the cadavers at the time of discovery that \( \text{H}_2 \) was generated after death by putrefaction.

As shown in Table. 2, in case 3, 1-propanol, employed as an indicator of putrefaction, and ethanol were detected in the blood at 0.02 mg/mL and 3.43 mg/mL, respectively. Jones et al. reported that the distribution frequency of ethanol concentrations in femoral venous blood in deaths of acute alcohol poisoning was investigated, and that the mean value was 3.60 mg/mL.\(^\text{24}\) The blood ethanol concentration shown in this case corresponds to the level of death from poisoning. Nanikawa et al. concluded that the ratio of ethanol to 1-propanol produced in postmortem blood was less than 20 times. If this quantitative relationship is applied to this case, it is estimated that the antemortem ethanol concentration is >3.03 mg/mL. However, other recent studies have reported the cases in which the concentration ratio of ethanol/1-propanol in postmortem blood exceeds 20 times,\(^\text{25,26}\) so that the total amount of ethanol may be derived from postmortem production in this case. Contrarily, in case 4, 1-propanol and ethanol were not detected, and in case 7, 1-propanol was not detected and ethanol was detected at 0.22 mg/mL. In case 7, the detection of ethanol may be misleading to distinguish between postmortem production and antemortem ingestion.

Thus, although the detection patterns of ethanol and 1-propanol varied in the three cases, \( \text{H}_2 \) was detected at high concentrations in any of the cases, and it was suggested that \( \text{H}_2 \) might be employed as an appropriate indicator of discrimination between postmortem production and antemortem ingestion.

However, future works should examine the relationship between putrefaction and \( \text{H}_2 \) production.
Furthermore, H2 is known to be produced by intestinal bacteria of indigestible carbohydrates, such as dietary fiber, and it is necessary to consider H2 concentration in the blood.

Conclusions

In this study, we examined the possibility of analyzing H2 by GC–SIM-MS using a single quadrupole instrument. H2 was analyzed by GC–MS in full scan mode using a TC-Molsieve 5A capillary column, and the H2 base peak at m/z 2 was observed. This suggested that it was possible to detect H2 by GC–MS. Therefore, the H2 analysis in SIM mode using double columns was performed, and a good peak shape that was largely bilaterally symmetrical was observed. Under the optimized conditions, the analysis of the H2 standard gas ranging from 5 to 1,000 ppm confirmed that the peak intensity of H2 exhibited an extremely high concentration dependence and proved that H2 could be analyzed by GC–SIM-MS. Additionally, we detected H2 in human blood by the developed GC–SIM-MS analysis using existing atmospheric 22Ne as the IS. The proposed GC–SIM-MS method exhibited high sensitivity in terms of LOD (1.7 ppm) and high repeatability in terms of the RSD (≥ 4.7%). A total of 11 blood samples from deceased individuals with unknown causes of death was determined quantitatively at H2 concentrations ranging from 5 to 905 ppm. Therefore, we concluded that the developed GC–SIM-MS method is extremely beneficial to the quantitative assay of H2 in biological samples and may also aid autopsy by analyzing the blood of the deceased as an indicator of putrefaction.
Supplementary material contains supporting calibration curve for detection of H₂ in air measured by GC-SIM-MS using ²²Ne as the internal standard. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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Table 1 Intraday and interday repeatabilities of the present method in whole blood specimens

| Spiked                | Intraday                     | Interday                     |
|-----------------------|------------------------------|------------------------------|
|                       | Concentration obtained (ppm)<sup>a</sup> | Repeatability (%)<sup>b</sup> | Concentration obtained (ppm)<sup>c</sup> | Repeatability (%)<sup>b</sup> |
| Low Concentration     | 99 ± 3.8                     | 5                             | 3.8                                      | 98 ± 4.6 | 4.7   |
| (100 ppm)             |                              |                              |                                         |         |      |
| High Concentration    | 492 ± 6.7                    | 5                             | 1.4                                      | 490 ± 14 | 2.9   |
| (500 ppm)             |                              |                              |                                         |         |      |

*Data are provided as mean ± standard deviation

*Relative standard deviation

*The measurements were made on three consecutive days with triplicate determinations of each
Table 2 H₂ concentration in whole blood samples of the 11 cadavers

| Case No. | Age (y) | Sex  | Concentration (ppm) | Cause of death | Other analytical observations |
|----------|---------|------|---------------------|----------------|--------------------------------|
| 1        | 77      | Male | 187                 | unknown        | Ethanol: 2.61 mg/mL 1-propanol: ND |
| 2        | 47      | Male | 29                  | unknown        | Ethanol: 2.89 mg/mL 1-propanol: ND |
| 3        | 48      | Male | 905                 | unknown        | Ethanol: 3.43 mg/mL 1-propanol: 0.02 mg/mL |
| 4        | 33      | Male | 15,059*             | jumping        | Ethanol: ND 1-propanol: ND |
| 5        | 30      | Female | 154               | hanging        | Ethanol: 1.34 mg/mL 1-propanol: ND |
| 6        | 49      | Male | 180                 | hanging        | Ethanol: trace 1-propanol: ND |
| 7        | 85      | Female | 10,768*            | unknown        | Ethanol: 0.22 mg/mL 1-propanol: ND |
| 8        | 39      | Male | 5                   | hanging        | Ethanol: ND 1-propanol: ND |
| 9        | 62      | Female | 21                | hanging        | Ethanol: trace 1-propanol: ND |
| 10       | 64      | Female | 142               | fire           | Carbon monoxide: 71% |
| 11       | 1       | Female | 181               | fire           | Carbon monoxide: 79% |

* Data are for reference purposes only.

ND not detectable
Figure Captions

**Fig. 1** a. TIC and extracted ion chromatogram of the H\textsubscript{2} standard gas (1\% in He) and b. Mass spectrum at 1.4 min. Conditions: capillary column, TC-Molsieve 5A (30 m × 0.32 mm); injection volume, 1.0 mL; oven temperature, 35 °C; monitoring mode, full scan (m/z 2–50).

**Fig. 2** SIM chromatogram of the H\textsubscript{2} standard gas (100 ppm in the air). Conditions: capillary column, TC-Molsieve 5A (30 m × 0.32 mm); injection volume, 1.0 mL; oven temperature, 35 °C; monitoring mode, SIM (m/z 2).

**Fig. 3** Effects of injection modes on H\textsubscript{2} (100 ppm in the air) detection by GC–SIM-MS. a Split (5:1), b splitless, and c direct. Conditions: capillary column, TC-Molsieve 5A (double column, 60 m × 0.32 mm); injection volume, 1.0 mL; oven temperature, 35 °C; monitoring mode, SIM (m/z 2).

**Fig. 4** Absolute calibration curve for detection of H\textsubscript{2} in air measured by GC–SIM-MS. The calibration range of H\textsubscript{2} was 5–1000 ppm and the peak area was used for the vertical axis.

**Fig. 5** Representative GC–SIM-MS chromatograms of the H\textsubscript{2} standard gas (100 ppm in the air). The double capillary column TC-Molsieve 5A (60 m × 0.32 mm) was employed. Other conditions were the same as those specified in Fig. 3.
**Fig. 6** GC–SIM-MS chromatograms of human whole blood spiked with 100 ppm H₂. The double capillary column TC-Molsieve 5A (60 m × 0.32 mm) was employed. Other conditions were the same as those specified in Fig. 3.

**Fig. 7** GC–SIM-MS chromatograms of (a) blank blood and (b) the whole blood sample of the deceased in case 1. The double capillary column TC-Molsieve 5A (60 m × 0.32 mm) was employed. Other conditions were the same as those specified in Fig. 3.
Fig. 1  a. TIC and extracted ion chromatogram of the H₂ standard gas (1% in He) and b. Mass spectrum at 1.4 min. Conditions: capillary column, TC-Molsieve 5A (30 m × 0.32 mm); injection volume, 1.0 mL; oven temperature, 35 °C; monitoring mode, full scan (m/z 2–50).
Fig. 2  SIM chromatogram of the H$_2$ standard gas (100 ppm in the air). Conditions: capillary column, TC-Molsieve 5A (30 m × 0.32 mm); injection volume, 1.0 mL; oven temperature, 35 °C; monitoring mode, SIM (m/z 2).
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Conditions: capillary column, TC-Molsieve 5A (double column, 60 m × 0.32 mm); injection volume, 1.0 mL; oven temperature, 35 °C; monitoring mode, SIM (m/z 2).
Fig. 4  Absolute calibration curve for detection of H₂ in air measured by GC–SIM-MS. The calibration range of H₂ was 5–1000 ppm and the peak area was used for the vertical axis.
Fig. 5  Representative GC–SIM-MS chromatograms of the H₂ standard gas (100 ppm in the air). The double capillary column TC-Molsieve 5A (60 m × 0.32 mm) was employed. Other conditions were the same as those specified in Fig. 3.
Fig. 6  GC–SIM-MS chromatograms of human whole blood spiked with 100 ppm H₂. The double capillary column TC-Molsieve 5A (60 m × 0.32 mm) was employed. Other conditions were the same as those specified in Fig. 3.
Fig. 7  GC–SIM-MS chromatograms of (a) blank blood and (b) the whole blood sample of the deceased in case 1. The double capillary column TC-Molsieve 5A (60 m × 0.32 mm) was employed. Other conditions were the same as those specified in Fig. 3.
