1. Introduction

Recently, biofuels have been developed as an alternative fuel to fossil hydrocarbons and as a solution to the problems of global warming. However, practical applications to internal combustion engines have been hindered by the deterioration of biofuel quality caused by impurities, hygroscopicity, and oxidation. Therefore, the quality of biofuels has been tightly regulated in many countries. In Japan, the sulfur content in liquid fuels is regulated by the Act on the Quality Control of Gasoline and Other Fuels, and standards are provided by the Japanese Industrial Standards (JIS). Similarly, water, methanol, sulfur, and copper contents in ethanol fuels are standardized by the JIS K 2190. The sulfur content in ethanol fuel is required to be under 10 mg kg$^{-1}$ as in gasoline and diesel oil. Certified reference materials (CRMs) are indispensable for the accurate analysis of sulfur in liquid fuels.

CRM5s for the quality control of biofuels are usually provided by national metrology institutes. For example, the biodiesel fuel CRMs, NIST SRM 2772 and SRM 2773, are provided by the National Institute of Standards and Technology (NIST, Geithersburg, MA, USA). Many components and physical characteristics of biodiesel fuel are certified. The Instituto Nacional de Metrologia, Normalizaçào e Qualidade Industrial (INMETRO, Rio de Janeiro, Brazil) has also issued a series of bioethanol CRMs with certified values for water content, copper content, sulfate content, density, and so on. However, no biofuel reference materials have been issued in Japan. Therefore, a certified reference material for bioethanol, NMIJ CRM 8301-a, has been developed by the National Metrology Institute of Japan (NMIJ). Concentrations of water, methanol, sulfur, and copper are certified in the CRM 8301-a.

This study describes the characterization of sulfur in CRM 8301-a by two methods, combustion followed by ultraviolet fluorescence detection and combustion ion chromatography detection. The certified sulfur value is 2.43 mg kg$^{-1}$ and the expanded uncertainty ($k = 2$) is 0.23 mg kg$^{-1}$.

Keywords
Bioethanol, Sulfur, Certified reference material, Quality control
Dimethyl sulfide was chosen as the sulfur compound for supplementation, because it is one of the sulfur compounds commonly formed by the fermentation and putrefaction of plant material. An amount of candidate reference material of approximately 3.6 kg was subdivided into 440 ampoules containing approximately 8.5 mL material. An amount of candidate reference material combusted to form sulfur oxides (SO\textsubscript{2}) was absorbed and oxidized to sulfate (SO\textsubscript{4}\textsuperscript{2–}) in the absorbing solution. The absorbing solution was 5 mL containing 1.7 mmol L\textsuperscript{–1} NaHCO\textsubscript{3}. The calibration solutions and standard addition solutions were prepared from thiocephene (benzene-free (99.5 %), Acros Organics, Geel, Belgium) and ethanol (reagent grade, Wako) solution. Hydrogen peroxide (atomic absorption spectrometry grade, Wako) solution was used as the absorbing solution for the CIC analysis. Standard phosphate ion solution (JCSS, Kanto Chemical Co., Inc., Tokyo, Japan) was added to the absorbing solution to monitor volume change.

2.3. Procedures for Measurement of Sulfur in the Candidate Reference Material

UVFL analysis used a total sulfur analyzer, TS-100V (Mitsubishi Chemical Analytech Co., Ltd., Chigasaki, Japan). In this method, sulfur dioxide (SO\textsubscript{2}) is formed by combustion of the sample, and SO\textsubscript{2} is then measured with the UVFL detector. Carrier gases were Ar and O\textsubscript{2}, and flow rates were 350 mL min\textsuperscript{–1} and 400 mL min\textsuperscript{–1}, respectively. Auxiliary gases were added during combustion of the sample, 150 mL min\textsuperscript{–1} of O\textsubscript{2} to combust the sample completely, and 150 mL min\textsuperscript{–1} of Ar to carry the products. Combustion tube temperature was 900 °C at the upper part and 1000 °C at the lower part. Sample injection volume and sample injection rate were 50 μL and 1.2 μL s\textsuperscript{–1}, respectively.

CIC analysis used the sample combustion unit of the TS-100V system. Combustion conditions were the same as for the UVFL analysis except for the sample injection volume (250 μL). Sulfur in the sample was combusted to form sulfur oxides (SO\textsubscript{3}). Then, SO\textsubscript{3} was absorbed and oxidized to sulfate (SO\textsubscript{4}\textsuperscript{2–}) in the absorbing solution. The absorbing solution was 5 mL of 0.03 % hydrogen peroxide containing 1 mg L\textsuperscript{–1} of phosphate ion as an internal standard. The combustion gas containing SO\textsubscript{2} was passed through the absorbing solution for 7 minutes. SO\textsubscript{4}\textsuperscript{2–} in the absorbing solution was measured with an ion chromatograph, DX320 (Dionex, Sunnyvale, CA, USA). The eluent was aqueous solution containing 1.8 mmol L\textsuperscript{–1} Na\textsubscript{2}CO\textsubscript{3} and 1.7 mmol L\textsuperscript{–1} NaHCO\textsubscript{3}. The flow rate was 1.5 mL min\textsuperscript{–1}. The sample injection volume was 100 μL. Two anion-exchangers, DIONEX Ion Pac AS4A-SC (4 × 250 mm) and AG4A-SC (4 × 50 mm) (Dionex, Sunnyvale, CA, USA), were utilized as the separation and guard columns, respectively. The column oven temperature was 30 °C. The effluent was monitored with a conductivity detector.

All standard solutions for the UVFL analysis were prepared by the gravimetric blending method using a 30 mL Alumi-Seal Vial with a Mininert Valve (GL Sciences Inc., Tokyo, Japan). The calibration solutions (2-3 mg kg\textsuperscript{–1}) were prepared by diluting thiophene with ethanol. The temperature, humidity, and atmospheric pressure in the laboratory were measured for buoyancy correction. The purity of the thiophene was evaluated by the freezing point depression method and the subtracting method at NMIJ\textsuperscript{7}). All sulfur solutions for the CIC analysis were prepared by the gravimetric blending method using a 10 mL Alumi-Seal Vial with Mininert Valve. Five levels (0-7.81 mg kg\textsuperscript{–1}) of sulfur solutions for standard addition were prepared from the NMIJ CRM 4217-a and toluene. Therefore, sulfur certified value in the CRM 8301-a is traceable to the International System of Units (SI). The sulfur solutions were mixed with the candidate reference material at a mass ratio of about 1 : 1 (for each 2.5 mL) to maintain constant ratio of ethanol and toluene, and sulfur content was measured by CIC analysis.

3. Results and Discussion

3.1. Homogeneity Assessment

The between bottle homogeneity of the candidate reference material was assessed by measuring 5 subsamples taken from 10 ampoules randomly selected from the 440 ampoules. Sulfur was determined by the UVFL method. Table 1 shows the results of the homogeneity assessment. The uncertainty associated with sample homogeneity was estimated by the method described in ISO Guide 35\textsuperscript{8}). The between bottle homogeneity standard deviation (s\textsubscript{bb}) and the effect of analytical variation on the standard uncertainty due to between bottle homogeneity (u\textsubscript{bb}) were 0.019 mg kg\textsuperscript{–1} and 0.005 mg kg\textsuperscript{–1}, respectively. Therefore, s\textsubscript{bb} = 0.019 mg kg\textsuperscript{–1} was adopted as the standard uncertainty due to inhomogeneity, because s\textsubscript{bb} was larger than u\textsubscript{bb}.

3.2. Stability Assessment

The long-term stability of the candidate reference material for one year was assessed before the certification. The evaluation of sulfur stability was performed using the UVFL method. The preservation conditions of the candidate reference material for the stability
assessments were room temperature (ca. 20 °C), 5 and −30 °C, respectively. The results of the stability assessment are summarized in Table 2. The effect of preservation temperature on stability was insignificant. Therefore, CRM 8301-a can be kept at room temperature. Uncertainty associated with sample instability was estimated using the results at room temperature in Table 2. The linear regression model was used to check for any trend in the data for the samples. ANOVA for linear regression showed the slope was not significant at the 5% level. Therefore, the standard uncertainty due to instability (\(u_{\text{lts}}\)) was calculated by Eq. (1) according to Eq. (6) in ref. 9). The larger value between \(t \times s(b_1)\) and \(t \times |b_1|\) was evaluated as \(u_{\text{lts}}\).

\[
u_{\text{lts}} = t \times s(b_1) \quad \text{or} \quad t \times |b_1| \quad (1)
\]

Where, \(b_1\) is the slope of the regression, \(s(b_1)\) is the standard deviation of the slope, and \(t\) is the period till the expiration date of certification. The expiration of NMIJ CRM 8301-a was set at 3.5 years from the determination of certified value, and the \(t\) value was \((3.5 \text{ year} \times 365)\) day. Based on this analysis of the stability test, the values of \(b_1\) and \(s(b_1)\) were \(8.43 \times 10^{-5}\) and \(7 \times 10^{-5}\), respectively. Because \(b_1\) was larger than \(s(b_1)\), \(u_{\text{lts}}\) was calculated using \(t \times b_1\), and was evaluated as 0.108 mg kg\(^{-1}\).

### 3.3. Analysis for the Determination of Certified Value

#### 3.3.1. Combustion Ultraviolet Fluorescence (UVFL) Method

Measurement by UVFL analysis was carried out by the external standard method. Three calibration solutions and five ampoules of the candidate reference material were measured by the UVFL method. Three calibration solutions were measured by the UVFL method before and after the measurement of samples (\(n = 5 \times 2\)). Sulfur contents in five ampoules were measured \((n = 5)\) at 2.431, 2.434, 2.426, 2.426, and 2.426 mg kg\(^{-1}\), respectively. Therefore, sulfur content by the UVFL (\(C_{\text{UVFL}}\)) was 2.434 mg kg\(^{-1}\) calculated as the mean of five results. Table 3 shows the uncertainty in sulfur content by the UVFL method. The standard uncertainty \(u(C_{\text{UVFL}})\) was 0.010 mg kg\(^{-1}\) that was calculated by combining the uncertainties due to the measurement, the raw materials of the calibration solutions, and the between bottle homogeneity of ampoules.

| Ampoule No. | 24 | 76 | 130 | 135 | 193 | 232 | 307 | 316 | 358 | 412 |
|-------------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| Sulfur content [mg kg\(^{-1}\)] | 2.416 | 2.477 | 2.433 | 2.416 | 2.401 | 2.392 | 2.443 | 2.463 | 2.431 | 2.478 |
| | 2.459 | 2.526 | 2.473 | 2.441 | 2.419 | 2.415 | 2.453 | 2.373 | 2.457 |
| | 2.452 | 2.476 | 2.407 | 2.437 | 2.396 | 2.394 | 2.418 | 2.403 | 2.406 | 2.424 |
| | 2.448 | 2.434 | 2.445 | 2.445 | 2.426 | 2.379 | 2.417 | 2.421 | 2.392 | 2.426 |
| | 2.420 | 2.405 | 2.445 | 2.430 | 2.386 | 2.393 | 2.420 | 2.459 | 2.413 | 2.440 |
| Average | 2.439 | 2.464 | 2.441 | 2.434 | 2.405 | 2.395 | 2.423 | 2.440 | 2.403 | 2.445 |
| SD | 0.019 | 0.046 | 0.024 | 0.011 | 0.016 | 0.014 | 0.011 | 0.026 | 0.022 | 0.023 |
| Total average | 2.43 |

| Test day | Room temperature | 5 °C | −30 °C |
|----------|------------------|------|--------|
| 0 (homogeneity study) | 2.43 | 2.42 | 2.45 |
| 53 (1.5 months) | 2.42 | 2.42 | 2.45 |
| 191 (6 months) | 2.40 | 2.39 | 2.39 |
| 253 (8 months) | 2.45 | 2.43 | 2.43 |
| 368 (12 months) | 2.46 | 2.46 | 2.44 |

| Source of uncertainty | Value | Standard uncertainty | Unit | Relative standard uncertainty |
|-----------------------|-------|----------------------|------|------------------------------|
| Measurement           | 2.434 | 0.006\(^a\)          | mg kg\(^{-1}\) | 0.0025 |
| Thiophene (starting material) | 1 | 0.0003 | 0.0003\(^b\) |
| Between ampoule       | 1 | 0.0031 | 0.0031 |
| Combined standard uncertainty | 2.434 | 0.010 | mg kg\(^{-1}\) | 0.0040 |

\(^a\) Standard uncertainty of the measurement was calculated according to Eq. (5.10) in ref. 10). \(^b\) Relative standard uncertainty of starting material of calibration standards was estimated by total sulfur in thiophene (38.09 ± 0.011)\(^\text{[\%]}\), which was decided at the development of NMIJ CRM 4215-a as shown in ref. 7).
The standard uncertainty (measurement number of standards, Eq. (5.12) in ref. 10). Addition method to cancel the matrix effect and the average of the weighted mean of the five measurements, and the measurement uncertainty was calculated as 0.043 mg kg\(^{-1}\). As a result, the standard uncertainty \(u(C_{\text{CIC}})\) was 0.048 mg kg\(^{-1}\), calculated by combining the uncertainties of the measurement and the concentration of CRM 4217-a of the standard addition solutions. Table 4 shows the uncertainty in sulfur content by CIC.

### 3.3.2. Combustion Ion Chromatography (CIC)

Measurement by CIC was carried out by the standard addition method to cancel the matrix effect and the effect of recovery efficiency. The sample solutions were prepared and measured independently five times on different days. The standard uncertainty \((S_{\text{std}})\) due to the regression was calculated by Eq. (2) according to Eq. (5.12) in ref. 10).

\[
S_{\text{std}} = \frac{s_{\text{std}}}{b} \left\{ \frac{1}{n} + \frac{\overline{y}^2}{b^2 \sum (x_i - \overline{x})^2} \right\}^{1/2}
\]

Where, \(b\) is the slope of the regression, \(\overline{x}\) and \(\overline{y}\) is the average of \(x_i\) (concentration of sulfur) and that of \(y_i\) (signal intensity), respectively, \(n\) is the total measurement number of standards, \(x_i\) is the concentration of standards, and \(s_{\text{std}}\) is the standard deviation of residual of \(y_i\), calculated by Eq. (3) according to Eq. (5.6) in ref. 10).

\[
s_{\text{std}} = \left\{ \frac{\sum (y_i - \hat{y}_i)^2}{n - 2} \right\}^{1/2}
\]

Where, \(\hat{y}_i\) is the estimated \(y_i\) value.

Results of the measurements were 2.374 ± 0.087 mg kg\(^{-1}\), 2.412 ± 0.080 mg kg\(^{-1}\), 2.390 ± 0.169 mg kg\(^{-1}\), 2.362 ± 0.071 mg kg\(^{-1}\) and 2.403 ± 0.133 mg kg\(^{-1}\), respectively. Based on these results, the sulfur content by CIC analysis \((C_{\text{CIC}})\) was 2.374 mg kg\(^{-1}\), calculated as the weighted mean of the five measurements, and the measurement uncertainty was calculated as 0.043 mg kg\(^{-1}\). As a result, the standard uncertainty \(u(C_{\text{CIC}})\) was 0.048 mg kg\(^{-1}\), calculated by combining the uncertainties of the measurement and the concentration of CRM 4217-a of the standard addition solutions. Table 4 shows the uncertainty in sulfur content by CIC.

#### 3.3.2. Combustion Ion Chromatography (CIC)

| Source of uncertainty | Value  | Standard uncertainty | Unit    | Relative standard uncertainty |
|-----------------------|--------|----------------------|---------|-----------------------------|
| Measurement           | 2.386  | 0.043                | mg kg\(^{-1}\) | 0.0182                      |
| CRM 4217-a (starting material) | 1      | 0.009                |         | 0.0090\(^{a)}\)           |
| Combined standard uncertainty | 2.386  | 0.048                | mg kg\(^{-1}\) | 0.0203                      |

\(\text{a) Relative standard uncertainty of starting material of standard addition solutions was estimated by the certified value of CRM 4217-a.}\)

#### 3.4. Calculation of Certified Value and Its Uncertainty

##### 3.4.1. Certified Value

The certified value of sulfur \((C_i)\) in the CRM 8301-a was determined as the weighted mean of the results of the UVFL and CIC analyses. Based on the results in Tables 3 and 4, the value of \(C_i\) was 2.426 mg kg\(^{-1}\).

##### 3.4.2. Uncertainty of Certified Value

The standard uncertainty of the certified value was evaluated using the uncertainties due to measurement \((u(C_{\text{chrm}}))\), inhomogeneity \((s_{\text{inh}})\) and long term stability \((t_{\text{stb}})\), where \(u(C_{\text{chrm}})\) was estimated including the uncertainty within methods \(u(C_{\text{wm}})\) and the uncertainty between methods \(u(C_{\text{bm}})\)\(^{8)}\). The value of \(u(C_{\text{wm}})\) was calculated as the weighted mean of the \(u(C_{\text{UVFL}})\) and \(u(C_{\text{CIC}})\). The \(u(C_{\text{bm}})\) was estimated by ANOVA using each measurement result. Therefore, the standard uncertainty of \(C_i\) was calculated by combining \(u(C_{\text{wm}})\), \(u(C_{\text{bm}})\), \(s_{\text{inh}}\) and \(t_{\text{stb}}\). The uncertainty of the certified value is summarized in Table 5. Thus, the expanded uncertainty 0.23 mg kg\(^{-1}\) was decided from the combined uncertainty, 0.114 mg kg\(^{-1}\) and the coverage factor, \((k = 2)\).

### 4. Conclusions

The NMIJ CRM 8301-a (bioethanol) was issued by NMIJ, AIST in March 2012. Certified components are water\(^{11)}\), methanol, sulfur and copper\(^{12)}\) which are regulated by JIS K 2190\(^{11)}\). Certified values are 1688 ± 26 mg kg\(^{-1}\) for water\(^{11)}\), 482 ± 23 mg kg\(^{-1}\) for methanol, 2.43 ± 0.23 mg kg\(^{-1}\) for sulfur, and 0.0537 ± 0.0041 mg kg\(^{-1}\) for copper\(^{12)}\), where plus or minus values are the expanded uncertainty. To determine the sulfur certified value, combustion ultraviolet fluorescence and combustion ion chromatography analysis were used. This CRM can be used for the calibration of instruments and for the confirmation of the validity of analytical
methods or instruments during analysis of bioethanol fuel samples and similar materials.

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要 旨

バイオエタノール認証標準物質における硫黄の検出

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産業技術総合研究所 計測標準総合センター（NMIJ, AIST）では、バイオエタノールの成分分析における精度管理用認証標準物質、NMIJ CRM 8301-aを開発した。開発した認証標準物質のマトリクスはコメを原料として製造されたエタノールである。認証項目は、水、メタノール、硫黄、銅の4成分であり、本認証標準物質はエタノール燃料中の成分分析における機器校正用および精度管理用に使用することができる。本論文では、硫黄の認証について報告する。硫黄の認証値は、アンプルに小分けした検査標準物質を分析して決定した。測定方法には、燃焼-紫外吸収光および燃焼-イオンクロマトグラフィーを適用した。硫黄の認証値は2.43 mg kg⁻¹、検量線法では0.23 mg kg⁻¹（k = 2）である。