Use of Relative Molar Sensitivity as a Specific Value for Evaluating Heptaoxyethylene Dodecyl Ether Concentrations in Methanol Solution

Miho KUROE,**† Masahiko NUMATA,* Naoko MASUMOTO,** Yuzo NISHIZAKI,** Naoki SUGIMOTO,** and Nobuyasu ITOH*

*Research Institute for Material and Chemical Measurement, National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan
**Division of Food Additives, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki, Kawasaki, Kanagawa 210-9501, Japan

Notes

Introduction

Non-ionic surfactants are widely used as detergents, emulsifiers, and dispersants, but they readily accumulate in aquatic environments. Since they have several homologs, because their structures feature hydrophobic and hydrophilic components, it is reasonable to quantify each surfactant using a representative surfactant, such as heptaoxyethylene dodecyl ether (HOEDE), as a calibrator, as described in the Waterworks Act of Japan for drinking water and Japanese Industrial Standard (JIS K 0102:2019) for industrial wastewater. Thus, the reliability of the HOEDE concentration in calibration solutions is important for accurately analyzing non-ionic surfactants. However, a reliable quantification of the HOEDE content in standard solutions is difficult because it is a highly hygroscopic agent with no specific absorption maximum in the UV-Vis region, and commercially available HOEDE reagents contain some homologs as impurities (Fig. S1).

Using a combination of ¹H quantitative NMR (qNMR) and HPLC (qNMR/HPLC) with an internal standard material that is traceable to the International System of Units (SI), the relative molar sensitivity (RMS) can be reliably calculated from the signal ratio of the analyte to internal standard material in the qNMR spectra and HPLC chromatograms. Because RMS can be directly obtained from the analyte and internal standard material measurement of a solution, it is unaffected by the hygroscopicity and volatility of the analyte. Furthermore, if the RMS is robust against changes in the analytical conditions, it can be considered to be a specific value under comparable analytical conditions. The use of RMS, obtained with a UV-Vis detector as a specific value, is emerging as a reliable and convenient strategy for quantifying the susceptible or precious target analytes. Thus, it should also be applicable for quantifying the HOEDE levels in calibration solutions. Although the use of a refractive index (RI) detector combined with HPLC (HPLC-RI) should be more suitable for a wider application range of compounds including HOEDE, there are no reports for estimating of RMS using HPLC-RI, and using it as a specific value. To use RMS obtained with HPLC-RI as a specific value, it is essential to examine the robustness against not only different conditions and systems, but also the long-term stability.

In this study, we calculated the RMS for HOEDE to an internal standard material for qNMR, namely 1,4-bis(trimethylsilyl)benzene-δ, (1,4-BTMSB-δ), via qNMR/HPLC-RI. We then examined its robustness against the RI cell temperature and the acetonitrile content in the mobile phase, HPLC system. Furthermore, the obtained HOEDE concentrations using a previously evaluated RMS were comparable to those obtained using a reference method for over 1 year.

Keywords Relative molar sensitivity (RMS), heptaoxyethylene dodecyl ether (HOEDE), qNMR, HPLC, RI detector

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at concentrations of approximately 1000 and 5000 mg L⁻¹, respectively. The calibration solution for HPLC (CalibHPLC) was prepared by diluting Calib NMR 10-fold with methanol.

A stock HOEDE solution (100 mg L⁻¹) was prepared by dissolving the reagent HOEDE in methanol, and then subsampled in brown glass ampoules. 1,4-BTMSB-d₄ was accurately weighed and dissolved in the stock solution to prepare sample solutions for examining the robustness of RMS for over 1 year.

Analytical systems

¹H qNMR analyses were performed using a 400-MHz NMR spectrometer (JNM-ECS400, JEOL, Tokyo, Japan) with a TH5-ATFG2D probe, as described previously with some modifications (Table S1). ¹H qNMR-RI experiments were conducted using three models: 1100-series (Agilent Technologies, Santa Clara, CA, USA), 1290-series (Agilent Technologies), and HLC-8320GPC (Tosoh Corporation, Tokyo, Japan). Separation was usually performed with an ODS column (ZORBAX Eclipse Plus C18, 2.1 mm i.d. x 150 mm, 1.8 μm; Agilent Technologies) at 30°C using a premixed 70% acetonitrile aqueous solution under the isocratic mode at a flow rate of 0.2 mL min⁻¹. The injection volume of the samples was 8 μL. HPLC measurements were repeated, and the results were expressed in the mean ± 2SD.

Results and Discussion

¹H NMR and HPLC results

Figure 1a presents the ¹H NMR spectrum of CalibNMR. These calibration solutions contain purified HOEDE and 1,4-BTMSB-d₄. The ¹H chemical shift was relative to methanol at 3.31 ppm. The peak intensities used for quantification were 0.13 for the alkyl residue of HOEDE and 12.9 for the methyl group of 1,4-BTMSB-d₄.

Calculation of RMS in the calibration solution and HOEDE concentration in sample solutions

RMS was calculated by using the molar ratio of HOEDE to 1,4-BTMSB-d₄ (R_{NMR}) in CalibNMR obtained via ¹H NMR, and the response ratio of HOEDE to 1,4-BTMSB-d₄ (R_{HPLC}) in CalibHPLC obtained via HPLC.

The mass fraction of HOEDE (Cₐ: mg kg⁻¹) was calculated using

\[ C_a = \frac{1}{R_{MS}} \times \frac{A_{sp}}{A_{sp}} \times \frac{M_r}{M_a} \times \frac{W_r}{W_S} \times P_r. \]

In this equation, \( A_{sp} \) and \( A_{sp} \) are the peak areas of HOEDE and 1,4-BTMSB-d₄, respectively, in the HOEDE sample solution obtained using HPLC. \( M_r \) and \( M_a \) are the molar masses (g mol⁻¹) of HOEDE and 1,4-BTMSB-d₄, respectively. \( W_r \) is the mass (mg) of 1,4-BTMSB-d₄. \( W_S \) is the mass (kg) of the stock HOEDE solution. \( P_r \) is the purity (kg kg⁻¹) of 1,4-BTMSB-d₄.

Comparison of RMSs for HOEDE between (a) the RI cell temperatures at 33 and 55°C, (b) the acetonitrile levels at 65 and 75%, and (c) among various HPLC-RI systems. (d) HOEDE concentrations on days 0, 195, and 401, as evaluated using RMS obtained on day 0 (filled symbols) in comparison with those obtained using the reference method (open symbols). Error bars indicate 2SD.
the signal at 0.1 – 0.5 ppm represented the methyl substitue of 1,4-BTMSB-d₄. In this study, the signals of the alkyl residue were used to calculate the molar ratio because they were not affected by the signal of methanol. The molar ratio obtained using qNMR (R_{qNMR}) was 0.0913 ± 0.0003 (n = 4). Figure 1b presents the HPLC chromatogram of CalibHPLC. Although no peaks originating from homologs were observed in the chromatograms, the response ratio of purified HOEDE to 1,4-BTMSB-d₄ changed slightly depending on the analytical conditions.

Robustness of RMS against different conditions

If RMS can be used as a specific value for HOEDE, it should be convenient for routine analytical usage. Although changes in the molar ratios obtained with different NMR systems and conditions should be negligible, because of its measurement procedure, there is a strong possibility that the intensity ratios using HPLC-RI should change depending on analytical conditions and systems. Thus, in this study, we examined the robustness of RMS against changes of the analytical conditions related to HPLC-RI, such as the RI cell temperature, acetontitrile content in the mobile phases, and differences in the analytical instruments and assessed its long-term stability.

First, we examined changes in the RMS at RI cell temperatures of 33 and 55°C, representing the minimum and maximum temperatures of the 1100-series system, respectively, using 70% acetonitrile as the mobile phase. The calculated RMS values were 1.73 ± 0.05 (n = 15) at 33°C and 1.75 ± 0.06 (n = 14) at 55°C (Fig. 2a). Then, the effects of the acetontitrile content in the mobile phase over the range of 65 – 75% were examined using the 1290-series and an RI cell temperature of 33°C. The calculated RMSs were 1.74 ± 0.05 (n = 7) and 1.71 ± 0.04 (n = 7) at 65 and 75% acetontitrile, respectively. Both values were comparable to the aforementioned value for 70% acetontitrile (Figs. 2a and 2b). Finally, the differences in the analytical systems were examined using three HPLC systems, and under the same separation conditions because the RI responses including the signal-to-ratio are strongly dependent on the HPLC-RI systems. The calculated RMSs were 1.74 ± 0.05 (n = 8), 1.73 ± 0.03 (n = 8), and 1.78 ± 0.13 (n = 7) for the 1100-series, 1290-series and HLC-8320GPC, respectively, and no significant differences were observed (Fig. 2c). The larger variation of the result for HLC-8320GPC was attributable to a five-times worse signal-to-noise ratio for this system, partly because of its unstable cell temperature at 35°C (limit of settable temperature). From these results, the RMS of HOEDE with 1,4-BTMSB-d₄ was considered to be robust against changes of the analytical conditions, possibly occurring during routine analyses.

We also examined the robustness of the RMS over a long period because gradual changes in the conditions of the analytical instruments over time are common. We examined the robustness of the RMS by quantifying HOEDE using the 1100-series and 1290-series because their results can be compared with those of another reliable reference method applied to calibrate the most upstream JCSS standard solution.

For the reference method, a UV detector that was not robust for HOEDE, but was more precise than RI detectors (data not shown), was applied, and the RMS obtained at each time point was used to calculate the HOEDE concentrations. For HPLC-RI, the RMS obtained in the day 0 analysis was applied for days 195 and 401, and the calculated HOEDE concentrations were 132.2 ± 3.6, 129.6 ± 4.4, and 130.5 ± 3.3 mg kg⁻¹ on days 0, 195 and 401, respectively (Fig 2d). The largest factor responsible for the variance was the repeatability of the HPLC measurements for all data, and no significant differences were observed among different days. Furthermore, the obtained HOEDE concentrations were comparable to both values and the repeatability obtained with the reference method (2.0 – 4.6% in 2SD). Thus, RMS can be used as a specific value and applied for the routine and reliable quantification of HOEDE levels in standard solutions.

Conclusion

In this study, we identified RMS as being a specific value via qNMR and HPLC-RI quantifying HOEDE with no absorption maximum over the UV and visible ranges. The RMS was robust against changes in the analytical conditions and stable for over 1 year. Although the applicable concentration should be somewhat high because of the lower sensitivity of RI detectors, the RMS value obtained with HPLC-RI should be useful for susceptible and precious analytes.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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