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Absolute Purity Determination of a Hygroscopic Substance, Indocyanine Green, Using Quantitative NMR (qNMR)

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Introduction

Quantitative NMR (qNMR) has emerged as a new absolute quantitation method for small molecules. In the Japanese Pharmacopoeia (JP), 15 compounds evaluated using qNMR are listed as HPLC analytical standards in the assay of crude drugs such as ginsenoside Rb 1 (GRB1), which is used as an analytical standard in the assay of crude drug section of the JP.1–4) In a previous study, we revealed that qNMR is applied to determine the absolute quantitative value of analytical standards for HPLC-based quantification. We have previously reported the optimal and reproducible sample preparation method for qNMR of hygroscopic reagents, such as saikosaponin a, which is used as an analytical standard in the assay of crude drug section of Japanese Pharmacopoeia (JP). In this study, we examined the absolute purity determination of a hygroscopic substance, indocyanine green (ICG), listed in the Japanese Pharmaceutical Codex 2002, using qNMR for standardization by focusing on the adaptation of ICG to JP. The purity of ICG, as an official non-Pharmacopoeial reference standard (non-PRS), had high variation (86.12 ± 2.70%) when preparing qNMR samples under non-controlled humidity (a conventional method). Additionally, residual ethanol (0.26 ± 0.11%) was observed in the non-PRS ICG. Next, the purity of non-PRS ICG was determined via qNMR when preparing samples under controlled humidity using a saturated sodium bromide solution. The purity was 84.19 ± 0.47% with a lower variation than that under non-controlled humidity. Moreover, ethanol signal almost disappeared. We estimated that residual ethanol in non-PRS ICG was replaced with water under controlled humidity. Subsequently, qNMR analysis was performed when preparing samples under controlled humidity in a constant temperature and humidity box. It showed excellent results with the lowest variation (82.26 ± 0.19%). As the use of a constant temperature and humidity box resulted in the lowest variability, it is recommended to use the control box if the reference ICG is needed for JP assays.

Key words quantitative NMR; hygroscopic substance; indocyanine green; absolute purity; humidity control; residual solvent

Quantitative NMR (qNMR) is applied to determine the absolute quantitative value of analytical standards for HPLC-based quantification. We have previously reported the optimal and reproducible sample preparation method for qNMR of hygroscopic reagents, such as saikosaponin a, which is used as an analytical standard in the assay of crude drug section of Japanese Pharmacopoeia (JP). In this study, we examined the absolute purity determination of a hygroscopic substance, indocyanine green (ICG), listed in the Japanese Pharmacopoeia 2002, using qNMR for standardization by focusing on the adaptation of ICG to JP. The purity of ICG, as an official non-Pharmacopoeial reference standard (non-PRS), had high variation (86.12 ± 2.70%) when preparing qNMR samples under non-controlled humidity (a conventional method). Additionally, residual ethanol (0.26 ± 0.11%) was observed in the non-PRS ICG. Next, the purity of non-PRS ICG was determined via qNMR when preparing samples under controlled humidity using a saturated sodium bromide solution. The purity was 84.19 ± 0.47% with a lower variation than that under non-controlled humidity. Moreover, ethanol signal almost disappeared. We estimated that residual ethanol in non-PRS ICG was replaced with water under controlled humidity. Subsequently, qNMR analysis was performed when preparing samples under controlled humidity in a constant temperature and humidity box. It showed excellent results with the lowest variation (82.26 ± 0.19%). As the use of a constant temperature and humidity box resulted in the lowest variability, it is recommended to use the control box if the reference ICG standard is needed for JP assays.

Quantitative NMR (qNMR) has emerged as a new absolute quantitation method for small molecules. In the Japanese Pharmacopoeia (JP), 15 compounds evaluated using qNMR are listed as HPLC analytical standards in the assay of crude drug section of the JP.1–4) In a previous study, we revealed that humidity affects the purity of hygroscopic reagents; moreover, humidity control before and during weighing is essential for a reproducible analysis, and indication of the absolute amount (not purity value), which is not affected by water content, is vital for hygroscopic product weight determination using qNMR.5,6) We have also determined an optimal and reproducible method for the sample preparation of hygroscopic marker compounds of crude drugs such as ginsenoside Rb 1 (GRB1), saikosaponin a (SSA), saikosaponin b 2 (SSB2), and barbaloin (BB) for qNMR. The results showed the importance of humidity control before and/or during weighing for qNMR.5,6) Namely, we examined the effect of humidity before and during weighing on the purity of commercial GRB1, with the purity value determined by qNMR; our results demonstrated the importance of humidity control. The standardization of humidity control before and during weighing for a specific time provides a practical approach for hygroscopic products, such as SSA and SSB2. For BB, humidity control for a specific time only before weighing is enough for determining purity in a reproducible manner.5,6) Recently, we studied the adaptation of indocyanine green (ICG), a fluorescence angiography agent (Fig. 1) listed in the

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ICG is a hygroscopic substance, and its official reference standard can be purchased from the Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ) for use as a non-Pharmacopoeial reference standard (non-PRS). The NMR spectrum of non-PRS ICG obtained after storing it in a drying chamber at 105 °C for 2 h revealed that it still contained residual ethanol (data not shown). This suggested that it is difficult to use non-PRS ICG as a reference standard because purity cannot be calculated using the mass balance method. Therefore, we aimed to use qNMR to directly determine its absolute purity, which is needed for its use as a HPLC reference standard in JP. We performed qNMR of ICG, with or without humidity control, using a saturated sodium bromide (NaBr) solution and a constant temperature and humidity box and compared the results obtained.

Results and Discussion

Non-controlled Humidity (Conventional Method) First, qNMR of non-PRS ICG was performed when preparing qNMR samples under uncontrolled humidity as a conventional method (Tables 1, 2 and Fig. 2a). The quantitation signals of ICG were examined. Non-PRS ICG had no interference signal when the signals around 6.5 ppm (position-3 and -5, -1 and -7) and around 4.2ppm (position-1” [2H × 2]) were used as the signals to be integrated (Fig. 2a). Therefore, these signals were used in the quantitation to calculate absolute purity. Additionally, the residual ethanol signals (CH₃-position, around 1.1 ppm and CH₂-position, around 3.5 ppm) were observed (Fig. 2a), and the CH₃-position signal was determined as the quantitation signal for ethanol, as it showed the sufficient peak intensity. Next, ICG purity and ethanol content were determined without humidity control before and during weighing in four laboratories (Labs A–D, Table 3). The humidity around the balance scale, monitored and recorded during weighing with a digital hygrometer in four laboratories, was 34–57%. The results showed a high variation in ICG purity (86.12 ± 2.70%) and ethanol content (0.26 ± 0.11%), which showed that the weight of hygroscopic ICG quickly changed depending on humidity. Consequently, the purity changed.

Controlled Humidity Using a Saturated NaBr Solution (the Saturated NaBr Solution Method) We next examined weight equilibration time of ICG to determine the appropriate duration of humidity control before weighing. At 25 °C and 57.6% humidity via water sorption-desorption analysis using thermal gravimetric analysis (TGA) equipment, the rate of weight change was less than 0.1% per hour after 5 h (Fig. S1). In addition, when qNMR of ICG was performed at 25 °C and 57.6% humidity using a saturated NaBr solution for 5 h, the ethanol signal almost disappeared (data not shown). Therefore, the humidity control condition for ICG was set at 5 h or more at 25 °C and 57.6% humidity. After adjusting non-PRS ICG under controlled humidity using a saturated NaBr solution for 5–19 h, the purity was re-determined using qNMR in five laboratories (Labs B, D–G, Table 4). The humidity around the electronic weighing balance, monitored and recorded during weighing with a digital hygrometer in five laboratories, was 25–46%. The purity of ICG under controlled humidity was 84.19 ± 0.47% with a lower variation (Table 4) than that under the uncontrolled humidity (Table 3). The standard deviation of
## Table 1. List of Devices, Parameters and Humidity Conditions for Sample Preparation

| Laboratory | A | B | C | D | E | F | G | H | I | J |
|------------|---|---|---|---|---|---|---|---|---|---|
| **Humidity condition** | (1) | (3) | (1) | (2) | (1) | (1) | (2) | (2) | (3) | (3) |
| Solvent | DMSO-d$_6$ 99.96 atom%D (Isotec) | DMSO-d$_6$ 99.9 atom%D (Wako) | DMSO-d$_6$ 99.9 atom%D (CIL) | DMSO-d$_6$ 99.9 atom%D (KANTO) | DMSO-d$_6$ 99.96 atom%D (Wako) | DMSO-d$_6$ 99.9 atom%D (Aldrich) | DMSO-d$_6$ 99.96 atom%D (Isotec) | DMSO-d$_6$ 99.96 atom%D (MERCK) | DMSO-d$_6$ 99.9 atom%D (MERCK) | DMSO-d$_6$ 99.9 atom%D (Isotec) |
| **Balance** | Ultramicro balance | Micro balance | Micro balance | Ultramicro balance | Micro balance | Ultramicro balance | Micro balance | Ultramicro balance | Micro balance | Ultramicro balance |
| Minimum indicated value (mg) | 0.0001 | 0.001 | 0.001 | 0.0001 | 0.001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Minimum weight (mg) | 0.2797 | 0.7843 | 1.398 | 1.033 | 0.6535 | 0.4881 | 1.398 | 1.886 | 0.8692 | 0.2271 |
| Calibration standard: DSS-d$_6$ (mg) | 0.9997 | 1.1745 | 2.002 | 2.145 | 2.130 | 1.0358 | 1.0421 | 1.687 | 2.573 | 0.9989 |
| Sample volume analyte: Iodocyanine Green (ICG) (mg) | 9.7137 | 10.0022 | 20.559 | 20.804 | 19.411 | 10.1037 | 9.8907 | 12.161 | 14.259 | 10.0014 |
| Added solvent volume (mL) | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| Humidity-controlling time (h) | — | 5 | — | 15 | — | 5 | 15 | 19 | 5 | 5 |

(1) Uncontrolled humidity, (2) controlled humidity using a saturated NaBr solution, and (3) controlled humidity in a constant temperature and humidity box

## Table 2. List of Devices and Parameters in 1H-qNMR

| Laboratory | A | B | C | D | E | F | G | H | I | J |
|------------|---|---|---|---|---|---|---|---|---|---|
| **Humidity condition** | (1) | (1) | (1) | (1) | (1) | (2) | (2) | (2) | (2) | (2) |
| **Spectrometer frequency** | 600 MHz | 600 MHz | 600 MHz | 600 MHz | 600 MHz | 600 MHz | 600 MHz | 600 MHz | 600 MHz | 600 MHz |
| **Probe type** | Normal | Cryogenic | Cryogenic | Normal | Normal | Cryogenic | Normal | Normal | Normal | Normal |
| **Spectral width** | 20 ppm | 20 ppm | 20 ppm | 20 ppm | 20 ppm | 25 ppm | 20 ppm | 20 ppm | 20 ppm | 20 ppm |
| **Digital filter** | ON | ON | ON | ON | ON | ON | ON | ON | ON | ON |
| **Pulse angle** | 90° | 90° | 90° | 90° | 90° | 90° | 90° | 90° | 90° | 90° |
| **Resolution** | 0.25 Hz | 0.25 Hz (0.125 Hz) | 0.25 Hz | 0.23 Hz | 0.125 Hz (2) | 0.25 Hz | 0.25 Hz | 0.25 Hz | 0.25 Hz | 0.25 Hz |
| **Relaxation delay time** | 60 s | 60 s | 60 s | 60 s | 60 s | 60 s | 60 s | 60 s | 60 s | 60 s |
| **Measurement temperature** | 24 °C | 25 °C | 27 °C | 25 °C | 23 °C(2) | 30 °C | 25 °C | 25 °C | 25 °C | 25 °C |
| **13C decoupling** | ON | ON | OFF | ON | ON | ON | ON | ON | ON | ON |
| **Decoupling sequence** | MPF8 | MPF9 | NA | MPF8 | MPF9 | MPF8 | MPF8 | MPF8 | MPF8 | MPF8 |
| **Scan times** | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 |
| **Dummy scan times** | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 |

(1) Uncontrolled humidity, (2) controlled humidity using a saturated NaBr solution, and (3) controlled humidity in a constant temperature and humidity box

NA: Not applicable
SSA qNMR analyses is less than 0.1% \((n = 3)\) under controlled humidity before and during weighing was carried out, as presented in our earlier study, \(^6\) and in the present study timed humidity control before weighing only was carried out, which may be the reason of a bigger variation for ICG. The ethanol content was significantly decreased in two labs (Labs E and F), and the signal was below the detection limit in three labs (Labs B, G, D) (Fig. 2b, Table 4). These results suggested that the residual ethanol in non-PRS ICG was replaced with water under controlled humidity.

Controlled Humidity in a Constant Temperature and Humidity Box  Subsequently, we examined the determination of ICG purity using a constant temperature and humidity box, which is a developed humidity control system, a balance within the box. The humidity control condition for ICG was set as 5 h at 25°C and 57.6% humidity, which were the same as the saturated NaBr solution method. qNMR analysis was performed under controlled humidity in the box in five laboratories (Labs A, E, H–J), which showed good results with a lower variation (82.26 ± 0.19%) (Table 5) than that of the saturated NaBr solution method (Table 4). Additionally, the ethanol signal in the qNMR spectra was below the detection limit in all the five labs (Fig. 2c).

The plausible reasons why the same purity for ICG could not be obtained using these two methods (a saturated NaBr solution with controlled humidity, and a constant temperature and humidity box with controlled humidity) are as follows: 1) We did not perform temperature and humidity calibration of the box and used the indicated humidity on the box, so the actual humidity might have been different; 2) we humidified

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**Table 3.** Purities (%) of ICG and Ethanol Content under Uncontrolled Humidity in Four Laboratories

| Position | Laboratory | Average (%) | S.D. (%) |
|----------|------------|-------------|----------|
| ICG      | A          | 84.41       | 0.09     |
|          | B          | 86.30       | 0.09     |
|          | C          | 90.39       | 0.52     |
|          | D          | 85.01       | 0.55     |
|          | Average    | 86.53       | 2.69     |
| Ethanol  | A          | 0.16        | 0.00     |
|          | B          | 0.25        | 0.00     |
|          | C          | 0.41        | 0.02     |
|          | D          | 0.22        | 0.00     |
|          | Average    | 0.26        | 0.11     |

S.D.: Standard deviation

**Table 4.** Purities (%) of ICG and Ethanol Content under Controlled Humidity Using a Saturated NaBr Solution in Five Laboratories

| Position | Laboratory | Average (%) | S.D. (%) |
|----------|------------|-------------|----------|
| ICG      | E          | 84.15       | 0.17     |
|          | B          | 83.62       | 0.04     |
|          | F          | 84.40       | 0.21     |
|          | G          | 84.46       | 0.07     |
|          | D          | 83.94       | 0.04     |
|          | Average    | 84.11       | 0.35     |
| Ethanol  | A          | 0.004       | 0.000    |
|          | B          | ND          | ND       |
|          | C          | 0.005       | ND       |
|          | D          | ND          | ND       |
|          | Average    | ND          | ND       |

ND: Not detected, S.D.: Standard deviation

**Table 5.** Purities (%) of ICG under Controlled Humidity in a Constant Temperature and Humidity Box in Five Laboratories

| Position | Laboratory | Average (%) | S.D. (%) |
|----------|------------|-------------|----------|
| ICG      | A          | 82.40       | 0.10     |
|          | H          | 82.58       | 0.21     |
|          | E          | 82.07       | 0.04     |
|          | I          | 81.97       | 0.04     |
|          | J          | 81.99       | 0.04     |
|          | Average    | 82.20       | 0.27     |
| Ethanol  | A          | 82.36       | 0.14     |
|          | H          | 82.52       | 0.11     |
|          | E          | 82.05       | 0.09     |
|          | I          | 82.10       | 0.04     |
|          | J          | 82.25       | 0.12     |
|          | Average    | 82.26       | 0.19     |

S.D.: Standard deviation

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ICG for 5 h or more using a saturated NaBr solution (57.6% humidity), but during weighing, ICG was exposed to a more dry environment (25–46%), and then this might have changed the purity of ICG.

Comparison of Absolute ICG Purities under Three Different Humidity Conditions The three different humidity conditions yielded comparable purity means of 86.12 ± 2.70%, 84.19 ± 0.47%, and 82.26 ± 0.19% under non-controlled humidity, controlled humidity using a saturated NaBr solution, and using a constant temperature and humidity box, respectively (Tables 3–5). As the results using the constant temperature and humidity box showed the lowest variability, it is recommended to use the control box if the reference standard of ICG is needed for JP assays. In addition, the box has an advantage of temperature and humidity control. Namely, by using it the temperature and humidity control of samples are straightforward, even during weighing. As the temperature and humidity control box has excellent operability, providing an accurate and precise value, we think that the box provides the suitable environment to measure the purity of a hygroscopic compound using qNMR.

Experiments

Table 1 summarizes the list of devices, parameters and humidity conditions for sample preparation in each lab, and Table 2 shows the list of devices and parameters for 1H-qNMR measurement in each lab.

Facilities A total of 10 investigators from 10 laboratories (A–J) performed an experiment separately.

Reagent, Reference Standards for qNMR and Solvents

ICG was purchased from PMRJ. DSS-d$_6$ (2,2-dimethyl-2-silapentane-5-sulfonate-d$_6$ sodium salt, MW = 224.36), a certified reference material (traceable to National Metrology Institute of Japan/National Institute of Advanced Industrial Science and Technology (NMIJ/AIST)), was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and used as the calibration standard for qNMR. Dimethyl sulfoxide-d$_6$ (DMSO-d$_6$) (>99.9% atom% D), used as a solvent for qNMR, is shown in Table 1.

Instruments and Equipment

The ultra-micro balance and the micro balance with the readability of 0.0001 mg and 0.001 mg were used, respectively (Table 1). Five 600 MHz NMR spectrometers equipped with a cryogenic probe, and three 600 MHz, one 500 MHz and two 400 MHz NMR spectrometers equipped with a normal probe were used for qNMR measurement (Table 2).

Preparation of Sample Solution

NMR Validation Test

Approximately 10–20 mg of each reagent and about 1–2.6 mg of reference standard for qNMR, which were precisely weighed and placed in the same vial together for each tare, were dissolved in the NMR solvent (DMSO-d$_6$) (1–2 mL). Of the sample solution, precisely 0.6 mL was sealed in an NMR sample tube (Table 1).

Humidity Control Conditions

(1) Non-humidity control condition: Sample weighing was performed in uncontrolled humidity. The observed humidity in the laboratory was 34–57%.

(2) Humidity control using a saturated NaBr solution: ICG was equilibrated for at least 5 h in a sealed container with a saturated NaBr solution at 25 °C under controlled humidity (57.6%). Sample weighing was performed in uncontrolled humidity with a saturated NaBr solution.

(3) Humidity control in a constant temperature and humidity box: FS-7110CTH (JEOL Ltd., Tokyo, Japan), a constant temperature and humidity system, was used. ICG was equilibrated for 5 h in a constant temperature and humidity box at 25 °C under controlled humidity (57.6%). Sample weighing was also performed under controlled humidity indicated on the box.

DSS-d$_6$ was equilibrated for 30 min or more under each condition (1–3) before weighing, then weighing under the same condition (1–3).

Conditions for qNMR Table 2 shows the list of devices and parameters for 1H-qNMR measurement in each lab. A reference standard for qNMR (DSS-d$_6$) was also used as the chemical shift reference signal (0 ppm). The δ value was expressed in ppm. The observed spectrum width was 20–25 ppm. A digital filter was used. The center of the spectrum was set at 5 ppm. The pulse width was set to the time at which a 90-degree pulse was obtained. Acquisition time was 4 s; the digital resolution was 0.125 or 0.25 Hz, and the delay time was 60 s. An auto FG shim or a Topshim was used for shim adjustment. The determination temperature was set at 23–30 °C. 13C decoupling with MP8 or MP9 was performed, except for Lab. C. The scan was performed 64 times, and the dummy scan was performed twice or four times. In principle, the measurement was performed three times for each sample following the internal standard method (AQARI: Accurate quantitative NMR with internal reference substance) to ensure that the S/N of the quantitative signal was 100 or higher. Alice 2 for qNMR, Purity Pro, Delta (JEOL Ltd.), TopSpin (Bruker, MA, U.S.A.), and VnmrJ 4.2 A (Agilent Technologies, CA, U.S.A.) were used for NMR data processing. The trimethylsilyl peak of the reference standard for qNMR (DSS-d$_6$) was set at 0 ppm. Phase correction and baseline correction were performed manually. The integration range for each peak was determined manually. All integrated values in this study were expressed in terms of purity (%). The purity of the reagents was calculated using the following formula based on a previous study 8–11):

\[
P_{\text{sample}} = \left( \frac{I_{\text{sample}}}{I_{\text{std}}} \times \frac{H_{\text{sample}}}{H_{\text{std}}} \times \frac{W_{\text{sample}}}{W_{\text{std}}} \times \frac{M_{\text{sample}}}{M_{\text{std}}} \right) \times P_{\text{std}}
\]

\(I = \) signal area, \(H = \) number of protons, \(W = \) weight,

\(M = \) molecular weight, \(P = \) purity (%),

\(\text{std}, \text{sample} = \) reference standard for qNMR and sample

The following numbers were used for the calculation: The number of protons of methyl groups in DSS-d$_6$ (reference standard for qNMR), CH$_3_\times_3 = 9$; molecular weight of DSS-d$_6$ = 224.36; molecular weight of ICG = 774.96 (C$_{35}$H$_{52}$N$_2$NaO$_6$S$_2$); and molecular weight of ethanol = 46.069 (C$_2$H$_6$O).

TGA Equipment The thermogravimetric analyzer TGA Q5000SA (TA Instruments) was used under following conditions: purge gas, nitrogen; feed rate in a humidity chamber, 200 mL/min; balance gas, 10 mL/min; sample weight, 5 mg; sample pan, metalized quartz sample pan; temperature in eight equilibration determination, 25 °C; and relative humidity, 57.6%.
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Conflict of Interest  The authors declare no conflict of interest.

Supplementary Materials  The online version of this article contains supplementary materials.

References
1) The Ministry of Health, Labour and Welfare, Japan. “The Japanese Pharmacopoeia Seventeenth Edition (2016).” <https://www.mhlw.go.jp/file/06-Seisakujouhou-11120000-Iyakushokuhinkyoku/JP17REV1.pdf>, cited 10 March, 2020.
2) The Ministry of Health, Labour and Welfare, Japan. “Supplement I to The Japanese Pharmacopoeia Seventeenth Edition (2017).”<https://www.mhlw.go.jp/content/11120000/000352508.pdf>, cited 10 March, 2020.
3) The Ministry of Health, Labour and Welfare, Japan. “Supplement II to The Japanese Pharmacopoeia Seventeenth Edition (2019).” <https://www.mhlw.go.jp/content/11120000/000597173.pdf>, cited 10 March, 2020.
4) The Ministry of Health, Labour and Welfare, Japan. “The Japanese Pharmacopoeia Seventeenth Edition (2016), General Information, Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy and its Application to Reagents in the Japanese Pharmacopoeia.”<https://www.pmda.go.jp/files/000217666.pdf>, cited 10 March, 2020.
5) Uchiyama N., Hosoe J., Miura T., Sugimoto N., Ishizuki K., Yamada Y., Iwamoto Y., Suematsu T., Komatsu T., Maruyama T., Igarashi Y., Higano T., Shimada N., Goda Y., Yakugaku Zasshi, 140, 1063–1069 (2020).
6) Uchiyama N., Hosoe J., Miura T., Sugimoto N., Ishizuki K., Yamada Y., Iwamoto Y., Suematsu T., Komatsu T., Maruyama T., Igarashi Y., Higano T., Shimada N., Goda Y., Chem. Pharm. Bull., 69, 26–31 (2021).
7) Pharmaceutical and Medical Device Regulatory Science Society of Japan, “Japanese Pharmaceutical Codex 2002,” Jiho Inc., Tokyo, 2002, pp. 128–129.
8) Ohtsuki T., Sato K., Abe Y., Sugimoto N., Akiyama H., Talanta, 131, 712–718 (2015).
9) Tada A., Takahashi K., Sugimoto N., Suematsu T., Arifuku K., Saito T., Ihara T., Yoshida Y., Ishizuki K., Nishimura T., Yamazaki T., Kawamura Y., Shokuhin Eiseigaku Zasshi, 51, 205–212 (2010).
10) Sugimoto N., Tada A., Suematsu T., Arifuku K., Saito T., Ihara T., Yoshida Y., Kubota R., Tahara M., Shimizu K., Ito S., Yamazaki T., Kawamura Y., Nishimura T., Shokuhin Eiseigaku Zasshi, 51, 19–27 (2010).
11) Sato-Masumoto N., Nishizaki Y., Saito N., Yamazaki T., Numata M., Ihara T., Sugimoto N., Sato K., Nippon Shokuhin Kagaku Gakkaishi Jpn. J. Food Chem. Safety, 24, 75–81 (2017).