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Development of a Chloroform Reference Material for the Proficiency Testing of Hazardous Compounds in Commercial Consumer Chemical Products Under the Consumer Chemical Products and Biocide Safety Management Act (K-BPR)

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Abstract : In this study, a chloroform consumer chemical product (CCP) reference material (RM) is successfully developed, with potential to be used in the proficiency testing of hazardous compounds in CCPs for analysis and testing agencies. Validation experiments are rigorously conducted to evaluate whether the RM meets the requirements set by the ISO 13528 and ISO Guide 35, using a reliable GC/MS method for the analysis of chloroform. The obtained calibration plot linearity, limit of detection (LOD), and limit of quantitation (LOQ) are excellent. The developed RM meets the homogeneity and stability requirements; the between-unit (s_{bb}) and within-unit (s_{wb}) standard deviations are less than 2.5%, and the stability is found to be guaranteed for 50 days.

Keywords: chloroform, reference material, GC/MS, consumer chemical products, biocides

Introduction

Consumer chemical products (CCPs) provide substantial benefits to human life, and thus their use is steadily growing in modern societies. However, their misguided use can potentially cause significant harm to the health of the public in general,[1-3] as demonstrated in the devastating incident in Korea, where biocidal chemicals such as polyhexamethylene guanidine (PHMG) and 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT), which were used as disinfectants for household humidifiers, were cosprayed with water into the air and inhaled, causing a large number of casualties.[4-9] In response to the public health concern arising from this incident, the Korean government legislated the CCPs and Biocides Safety Management Act (K-BPR) in 2018. According to this law, manufacturers or importers have to confirm whether their CCPs comply with the safety criteria, which dictates the allowed quantities for listed biocidal chemicals, through the submission of product analysis/testing results.

Reference material (RM) is defined as "*a material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process*." In the quality assurance compliance process, the RM is often used by analysis and testing agencies, particularly for the validation of analytical methods, calibration of instruments, and proficiency testing (PT). In particular, an RM or certified RM (CRM) is needed in the PT for the analysis and testing agencies that will have an accredited authority to issue quantitative analysis/test results.[10–13] However, to the best of our knowledge, there is no commercially available CCP RM (or CRM) for the quantitative analysis of listed chemicals in CCPs. One of the chemicals listed in K-BPR as a hazardous substance, particularly when used in CCPs, is chloroform, which is suspected of causing cancer, according to the International Agency for Research on Cancer monographs.[14,15] Motivated by this, we were interested in developing a CCP RM containing chloroform to facilitate the determination of the chloroform concentration in disinfectants. This study describes the preparation of a chloroform-containing RM, along with validation experiments such as assessment of homogeneity and stability performed following the ISO Guide 35 for the developed (disinfectant) CCP RM.

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Materials and Methods

Materials and chemicals

Chloroform, chloroform-*d* (deuterated chloroform, internal standard), and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other organic solvents used in the experiment were of HPLC-grade, and purchased from Daejung Chemicals & Metals (Siheung, Korea). Syringe filters were purchased from Agilent Technologies (Palo Alto, CA, USA).

Preparation of RM and calibration curve

As the matrix CCP, an anonymous disinfectant product (in a liquid form) for infants was purchased from the online market, for which the absence of chloroform was experimentally confirmed. In the K-BPR act enforced by the Korean Ministry of Environment, the allowed concentration of chloroform in a commercial disinfectant is 30 mg/kg.[16] Thus, the concentration of chloroform in the developed RM was set to be close to this concentration, that is, 40 mg/kg, in the present study.

Two stock solutions of chloroform and chloroform-*d* with a concentration of 1,000 mg/L were prepared by adding 100 mg of the original solution to methanol in a 100 mL volumetric flask. Due to the high volatility of chloroform and chloroform-*d*, the 100 mL volumetric flask was first filled with methanol, and then placed on an electric balance while adding chloroform. The chloroform RM with a chloroform concentration of approx. 40 mg/L was prepared by initially pipetting 4 mL of the chloroform stock solution (approx. 4 mg) to a 100 mL volumetric flask, followed by the addition of the anonymous disinfectant solution to reach 100 mL. Three RMs were prepared following this method, and the average density was determined to be 1.00 g/mL; this density was used later for the conversion of the unit from mg/L to mg/kg. The prepared RM was further diluted 10-fold using methanol. Since chloroform has a low solubility in the RM solution, it was necessary to add methanol to the original RM solution. Furthermore, the dilution of the RM solution could minimize an interfering peak in the chromatogram, which was presumably due to the matrix effect. To prepare a 10-fold diluted RM containing 5.0 mg/L internal standard chloroform-*d*, 1 mL of the initially prepared RM, and 50 μL of chloroform-*d* stock solution were added to 8.95 mL of methanol in a 10 mL volumetric flask. Then, 5 mL of the diluted RM was transferred to a conical tube, into which 300 mg of ascorbic acid was added to remove residual chlorine in the disinfectant. These 10-fold dilution and chlorine removal steps were done for every stability and homogeneity assessments. The final RM solution was filtered using a polytetrafluoroethylene (PTFE) syringe filter (0.22 μm, pore size).

A calibration curve was constructed using the chloroform standard solutions with chloroform concentrations of 0.5, 2.5, 5.0, 7.5, and 10.0 mg/L, to which the internal standard chloroform-*d* at a concentration of 5.0 mg/L was added.

GC/MS-SIM (selected-ion monitoring) analysis

The chloroform standards and the chloroform RM were analyzed by GC/MS (G1530A/5973; Agilent Technologies, Santa Clara, CA, USA), using A DB-WAX column (30 m × 0.25 mm, 0.25 μm, Agilent Technologies). High purity helium gas was used as a carrier gas at a flow rate of 1.0 mL/min, and the sample inlet temperature was set at 200°C. The temperature in the transfer line, ion source, and quadrupole was set at 250°C, 230°C, and 150°C, respectively. The sample injection volume was 1 μL, and was split into a 1:10 ratio. The temperature gradient for GC was as follows: 40°C for 4 min, then heating up to 180°C at a rate of 10°C/min. After the gradient run, the post-run conditions for the standard solution and the RM were 180°C for 5 min and 240°C for 30 min, respectively. The following MS conditions were used for the analysis: mass range, *m/z* 35–200; solvent delay, 4.0 min; dwell time, 30 ms; SIM mode with *m/z* 83 and 85 for chloroform, and *m/z* 84 and 86 for chloroform-*d*.

Data analysis

The GC chromatographic peaks were identified, and their areas were integrated using the software Chemstation (F.01.01.2317; Agilent Technologies, Santa Clara, CA, USA) equipped with the NIST GC/MS library. After obtaining the chloroform calibration curve in the range of 0.5 to 10 mg/L, the concentration of the RM was calculated.

Validation

The linearity of the calibration curve was determined by calculating the coefficient of determination (R^2) of the curve. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the signal-to-noise ratios of 3 and 10, respectively.

Homogeneity assessment of the RM

The homogeneity and stability of the prepared RM were evaluated following two different ISO guidelines, *i.e.*, ISO Guide 35 and ISO 13528.[12,13] Both guidelines evaluate the sample homogeneity by measuring the within- (s_w) and between-unit (s_{bb}) standard deviations of the prepared samples to determine whether they satisfy the specified criteria. For example, the ISO Guide 35 suggests applying a one-way analysis of variance (or ANOVA test) to calculate the standard deviations, for which a 5% level of significance should be met.[17] In the ISO 13528 guideline, a comparison between the between-unit standard deviation, s_{bb}, with the standard deviation of proficiency assessment, $\hat{\sigma}$, is made, in which the homogeneity requirement of $s_{bb} \leq 0.3\,\hat{\sigma}$ should be met, where $\hat{\sigma}$ is set as 25% of the set concentration, 10 mg/kg. For the homogeneity test, a 100 mL RM solution was prepared, and it was divided into ten different bottles. Each bottle was further divided into two sample vials, and the concentration of each vial was measured in triplicate.

Stability monitoring of the RM

According to the ISO guidelines, there are two stability monitoring methods for RM, *i.e.*, simple stability monitoring and transportation stability monitoring, which evaluate the stabilities of the RM under the specified storage and transport conditions, respectively, for a certain period of time. We followed the simple stability monitoring approach suggested by the ISO Guide 35, according to which the RM was stored in a single storage condition.

The prepared chloroform RMs were stored under -20°C, and their stability monitoring was performed over 50 days. The concentration of the RM was measured in triplicate on Day 0, 1, 3, 5, 7, 10, 20, 30, 40, and 50. The stability assessment was carried out using two different methods suggested by the ISO guidelines ISO Guide 35 and ISO 13528. According to the ISO Guide 35, the stability evaluation of the RM is made by monitoring the slope of the linear regression plot for the measured concentrations at different days to determine the deviation of the slope from the value of 0. The degree of the slope deviation from 0 is evaluated statistically by a two tailed Student *t*-test at the 95% level of confidence. Meanwhile, the ISO 13528 guideline proposes the comparison between the general average of the concentration obtained in the homogeneity test and that obtained in the stability monitoring. The difference between the two values must be less than 0.3 $\hat{\sigma}$, where $\hat{\sigma}$ is the standard deviation for PT, and we set the value to be 25% of the set concentration.

Results and Discussion

Characteristics of the RM

The prepared RM was characterized using GC/MS analysis**.** Figure 1 shows the GC/MS-SIM chromatograms of the 10-fold diluted RM. For the SIM analysis, the chloroform isotopes with *m/z* 83 and 85, and those of chloroform-*d* at *m/z* 84 and 86 were monitored; the abundance ratio of these two isotopes is 3:2. In the subsequent analysis, the abundances of the two isotopes were summed. In the repeated measurements for the homogeneity and stability assessments, the peak shapes, retention times, and baselines of the chromatograms were found to be very consistent.

Calibration curve

As shown in Figure 2, the chloroform standard solutions at five different concentrations, *i.e.*, 0.5, 2.5, 5.0, 7.5, and 10 mg/L, containing 5.0 mg/L of internal standard were monitored using the GC/MS-SIM mode. For the monitoring of chloroform, two ion peaks at *m/z* 83 and 85 were selected (Figure 2(a) and (b)), whereas the other two peaks at *m/z* 84 and 86 were monitored for chloroform-*d* (Figure 2(c) and (d)). Figure 3 shows the calibration curve acquired

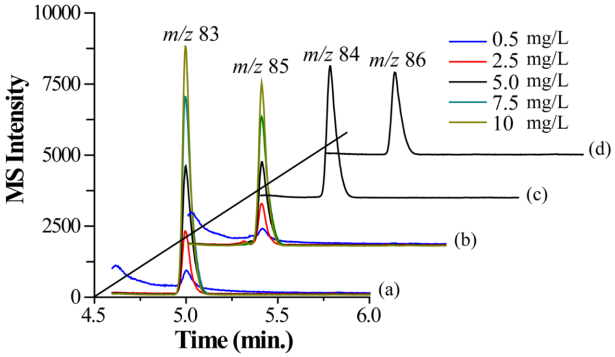

Figure 2. GC/MS-SIM chromatograms of the peak at *m/z* 83 (a) and *m/z* 85 (b) obtained for chloroform at five different concentrations, *i.e.*, 0.5, 2.5, 5.0, 7.5, and 10 mg/L. For reference, the SIM chromatograms of the peaks of chloroform-*d* at *m/z* 84 (c) and *m/z* 86 (d) are also shown.

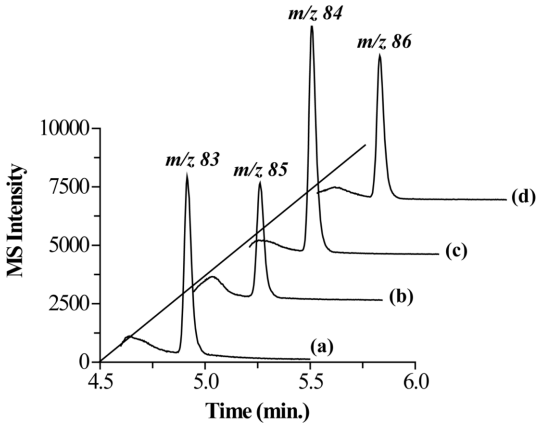

Figure 1. GC/MS-SIM chromatograms of the 10-fold diluted reference material for chloroform at *m/z* 83 (a) and *m/z* 85 (b) and for 5.0 mg/L chloroform-*d* at *m/z* 84 (c) and *m/z* 86 (d).

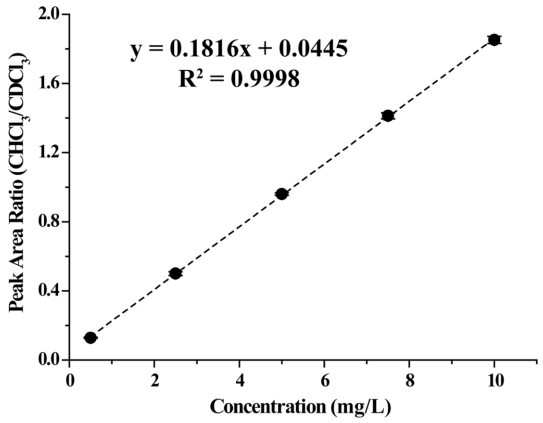

Figure 3. Calibration curve acquired for the quantitative analysis of chloroform in the developed reference material.

for the quantitative analysis of the RM, wherein the relative peak area of chloroform against that of chloroform-*d* at each concentration was plotted. The acquired calibration curve showed a good linearity (R^2 = 0.9998) in the selected concentration range. The LOD and LOQ for chloroform were determined to be 0.549 mg/kg and 1.812 mg/kg, respectively.

Assessment on the stability of the RM

Figure 4 shows the concentration of the RM measured over 50 days of the stability test. In the linear regression model, the slope of the stability plot tends to be close to 0 when the sample is stable enough to give consistent concentrations over the designated period of time. Table 1 shows the concentrations measured during the stability monitoring over 50 days. The average concentration of the RM used in the stability monitoring was 39.1 mg/kg, and the linear regression model gives a slope of -0.048, which is very close to the ideal value of 0. In the Student t-test suggested by the ISO Guide 35 to evaluate whether the acquired slope is statistically different from the value 0, the two tailed *t*-value was 0.047, with the theoretical *t*-value being 2.26 at a *p*-value < 0.05 and a degree of freedom of 8 (see Table 1). Since the sample *t*-value was lower than the theoretical value, it can be concluded that the slope of the linear regression plot did not statistically deviate from the value 0 with 95% confidence level.

In the ISO 13528 guideline, the average concentrations obtained from the homogeneity and stability tests are compared to evaluate whether the difference between the two average values is significant. In this study, we set the standard deviation of the proficiency test ($\hat{\sigma}$) to be 25% of the set value 10 mg/kg. Accordingly, the difference between the general averages should be less than 0.3 $\hat{\sigma}$ to guarantee the stability of the RM. The difference between the two averages was determined to be 1.164 mg/kg, which was less than 0.3 $\hat{\sigma}$ (3 mg/kg). This result confirms that the chloroform CCP RM is stable over 50 days after its preparation.

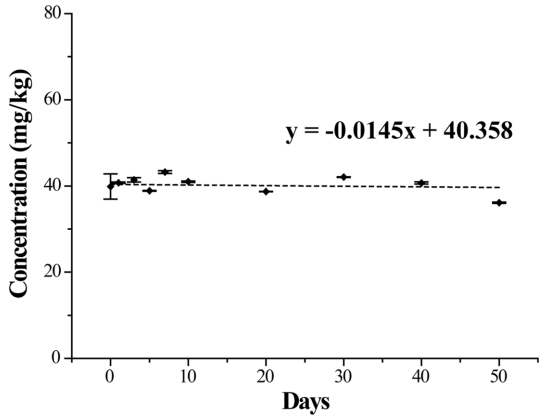

Figure 4. Stability of the reference material over 50 days and its linear regression fitting.

Assessment on the homogeneity of the RM

Table 2 presents the concentrations determined in the homogeneity assessment. The between-unit standard deviation (s_{bb}) and the within-unit standard deviation (s_w) determined by

Table 1. Stability assessment of the reference material

Day	Concentration (mg/kg)	Standard Deviation (mg/kg)
0	39.8	2.94
1	40.7	0.158
3	41.4	0.497
5	38.9	0.090
7	43.2	0.340
10	41.0	0.169
20	38.7	0.027
30	42.0	0.073
40	40.7	0.269
50	36.1	0.131
ISO Guide 35		
Slope	−0.048	
Theoretical *t*-value	2.26	σ = 0.05 / d.f. = 8
Sample *t*-value	0.047	
ISO 13528		
Mean of the samples in homogeneity test		39.1 mg/kg
Mean of the sample in stability test		40.2 mg/kg
Difference		1.16 mg/kg
0.3 σ		3.00 mg/kg

Table 2. Homogeneity assessment of the reference material

Sample	Measurement #1 (mg/kg)	Measurement #2 (mg/kg)	Mean (mg/kg)
1	37.8	38.8	38.3
2	41.7	38.6	40.2
3	38.5	37.9	38.2
4	40.4	40.2	40.3
5	38.9	38.4	38.6
6	38.8	38.5	38.7
7	38.7	38.9	38.8
8	39.8	40.9	40.3
9	39.2	40.5	39.9
10	37.2	38.2	37.7
standard deviation between bottles			0.738 mg/kg
standard deviation within bottle			0.896 mg/kg
Relative standard deviation between bottles			1.84%
Relative standard deviation within bottle			2.24%

the ISO guidelines were identical, 0.738 mg/kg (1.84%) and 0.896 mg/kg (2.24%), respectively. These values satisfy both criteria, indicating that the developed RM is homogeneous.

Conclusions

A chloroform CCP (disinfectant) RM was successfully developed with satisfactory validation results. The validation analyses were carried out using a reliable GC/MS method, in which the linearity of the calibration plot, LOD, and LOQ were examined. It was also shown that the developed RM meets the homogeneity and stability requirements suggested by the ISO guidelines ISO Guide 35 and ISO 13528. Specifically, in the homogeneity assessment, the between-unit (s_{bb}), and within-unit (s_{wb}) standard deviations were determined to be 1.84% and 2.24%, respectively, and the prepared RMs were very stable over 50 days from their initial production.

Acknowledgments

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References

1. Alavanja, M. C. R. *Rev. Environ. Health* **2010**, 24, 303.
2. Trantallidi, M.; Dimitroulopoulou, C.; Wolkoff, P.; Kephalopoulos, S.; Carrer, P. *Sci. Total Environ.* **2015**, 536, 903.
3. Kwon, K.-D.; Jo, W.-K.; Lim, H.-J.; Jeong, W.-S. *Environ. Sci. Pollut. Res.* **2018**, 15, 521.
4. Lee, J. H.; Kim, Y. H.; Kwon, J.-H. *Environ. Sci. Technol.* **2012**, 46, 2498.
5. Hwang, H. J.; Nam, J. J.; Yang, S. I.; Kwon, J.-H.; Oh, H. B. *Bull. Korean. Chem. Soc.* **2013**, 34, 1708.
6. Yoon, D. H.; Lee, D. K.; Lee, J. H.; Cha, S. W.; Oh, H. B. *Rapid Commun. Mass Spectrom.* **2015**, 29, 213.
7. Park, D.-U.; Friesen, M. C.; Roh, H.-S.; Choi, Y.-Y.; Ahn, J.-J.; Lim, H.-K.; Kim, S.-K.; Koh, D.-H., Jung, H.-J.; Lee, J.-H.; Cheong, H.-K.; Lim, S.-Y.; Leem, J.-H.; Kim, Y.-H.; Paek, D.-M. *Indoor Air* **2015**, 25, 631.
8. Kim, H.-R.; Hwang, G.-W.; Naganuma, A.; Chung, K.-H. *J. Toxic. Sci.* **2016**, 41, 711.
9. Bae, J. U.; Park, M. J.; Lee, J. M.; Song, I. S.; Joo, Y. H.; Lee, C. S.; Kwon, J. H.; Moon, B. J.; Oh, H. B. *Int. J. Mass Spectrom.* **2019**, 435, 298.
10. Kupiec, K.; Konieczka, P.; Namieśnik, J. *Crit. Rev. Anal. Chem.* **2009**, 39, 311.
11. Kiełbasa, A.; Gadzała-Kopciuch, R.; Buszewski, B. J. *Crit. Rev. Anal. Chem.* **2016**, 46, 224.
12. International Organization for Standardization. Statistical methods for use in proficiency testing by interlaboratory comparisons, ISO 13528, 1st ed.. International Organization for Standardization: Geneva, Switzerland, **2005**.
13. International Organization for Standardization, Reference materials–Guidance for characterization and assessment of homogeneity and stability, ISO Guide 35, 4th ed.. International Organization for Standardization: Geneva, Switzerland, **2017**.
14. Fiss, E. M.; Rule, K. L.; Vikesland, P. J. *Environ. Sci. Technol.* **2007**, 41, 2387.
15. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 98, International Agency for Research on Cancer: Lyon, France, **2007**.
16. National Institute of Environmental Research. Regulation on the Criteria and Methods of Testing and Inspection of the Safety Confirmation Targeted Consumer Chemical Products, Vol. 2018-71, National Institute of Environmental Research: Incheon, South Korea, 2018.
17. Kim, J.-H.; Choi, S.-G.; Oh, Y.-G.; Hong, S.-M.; Kim, S.-B.; Woo, I.-D.; Kim, J.-Y.; Seo, J.-S. *Korean J. Environ. Agric.* **2016**, 35, 223.

