Regular Article

Validation Study for Establishing a Standard Test Method for Volatile Organic Compounds in Indoor Air in Japan using Solvent Extraction

Masahiro Chiba,[a] Shiori Oizumi,[a] Aya Onuki,[b] Ikue Saito,[b] Reiko Tanaka,[c] Takashi Yamanouchi,[c] Yuko Yokoyama,[d] Takanari Wakayama,[e] Hiroyuki Ohno,[e] Maiko Tahara, [f,*] and Shinobu Sakai [f]

[a]Hokkaido Institute of Public Health; Kita 19, Nishi 12, Kita-ku, Sapporo, Hokkaido 060-0819, Japan; [b]Tokyo Metropolitan Institute of Public Health; 3-24-1, Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan; [c]Yokohama City Institute of Public Health; 2-7-1, Tomiokahigashi, kanazawa-ku, Yokohama, Kanagawa 236-0051, Japan; [d]Chiba Prefectural Institute of Public Health; 666-2, Nitona-cho, Chuo-ku, Chiba, Chiba, 260-8715, Japan; [e]Nagoya City Public Health Research Institute; 4-207, Sakurazaka, Moriyama-ku, Nagoya, Aichi 463–8585, Japan; [f]National Institute of Health Sciences; 3-25-26, Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

> The Ministry of Health, Labour and Welfare of Japan has set the guideline values for indoor air concentrations of 13 volatile organic compounds (VOCs) and semi-volatile organic compounds from 1997 to 2002. However, in 2019, the guideline values for three of these substances, including xylene, were revised and regulated more strictly. Additionally, the manual for analysis of VOCs in indoor air, established in 2001 by the Committee on Sick House Syndrome: Indoor Air Pollution, has not been updated for over 20 years. In this study, we confirmed that the current analytical method for VOCs in indoor air using solvent extraction which was established in 2001, is applicable to VOCs that have been revised or added since then. We proposed it as a standard test method and performed an inter-laboratory validation study in five laboratories to prove this. This validation study included nine substances: six VOCs with current guideline values and three VOCs as candidates for newly setting guideline values. Additional amount in this study was set as 1 μg, less than one-tenth of the guideline value for xylene. The results showed that the average recovery, repeatability, and reproducibility for the nine substances in the five laboratories were 75.4%–115%, 0.78%–9.6%, and 3.6%–21%, respectively. These values satisfied the determined criteria ranges, suggesting that our proposed analytical method can be used as a standard test method.

> **Key words** indoor air, volatile organic compounds, inter-laboratory validation, solvent extraction, standard test method

INTRODUCTION

Construction materials like wallpapers, paints, glues, and household products contain various types of chemicals and are the main source of volatile organic compounds (VOCs) in indoor environments.[1] In the 1990s, the improvement in the indoor living environment of Japan through various approaches, such as using high airtightness and thermal insulation in buildings, has led to health problems in residents, suspected to be caused by the contamination of VOCs in indoor air.[2,3] These health problems have been termed "sick building syndrome" or "sick house syndrome." Thus, to mitigate the health risks associated with indoor air chemicals, the Ministry of Health, Labour and Welfare (MHLW) of Japan set some guideline values for the indoor air concentrations of 13 VOCs and semi-volatile organic compounds from 1997 to 2002. Although the concentrations of these substances in indoor air decreased after implementing these measures, symptoms caused by alternative substances were reported.[4,5] Consequently, the guideline values were revised based on the latest knowledge and report of the Committee on Sick House Syndrome: Indoor Air Pollution (CIAP). In 2019, the guideline values of

three substances were lowered and regulated more strongly.[6] Additionally, CIAP proposed the analytical method "Manual for Measuring Chemical Substances in Indoor Air,"[7] which describes two methods to analyze VOCs in indoor air: solid-phase adsorption solvent extraction gas chromatography/mass spectrometry (SE method) and solid-phase adsorption thermal desorption gas chromatography/mass spectrometry (TD method). However, more than 20 years have passed since these methods were introduced, so it is important to determine a more efficient method based on the guideline values established after 2001.

Recently, we developed standard test methods for analyzing two phthalates, di-*n*-butyl phthalate and di-2-ethylhexyl phthalate, and three insecticides, fenobucarb, diazinon, and chlorpyrifos, whose guideline values had been previously established.[8–10] In the present study, we focused on the SE method to analyze VOCs in indoor air. The SE method is superior to the TD method in terms of not requiring any special equipment. To determine a versatile standard test method for the corresponding new guideline values of VOCs and other chemical substances, we conducted an inter-laboratory validation study using the SE method.

*To whom correspondence should be addressed. e-mail: tahara@nihs.go.jp

MATERIALS AND METHODS

Target Substances We included nine target substances in this study. Among the 13 substances for which the MHLW has set guideline values, 6 substances, toluene, xylene (including *o*-, *m*-, and *p*-isomers), styrene, ethylbenzene, *p*-dichlorobenzene, and tetradecane, classified as VOCs, were selected as target substances in this study. Additionally, we also selected 2-ethyl-1-hexanol, 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (TPMI), and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TPDI) as target substances. The guideline values for these three substances are currently under discussion by CIAP (Table 1).[11]

Chemicals and Equipment Toluene, *o*-, *m*-, and *p*-xylene (hereafter referred to as xylene), styrene, ethylbenzene, *p*-dichlorobenzene, 2-ethyl-1-hexanol, TPMI, and TPDI were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) as a mixed standard stock solution for indoor environment measurements. Guaranteed reagent and Wako special grade tetradecane were purchased from Kanto Chemical Co., Inc. and Fujifilm Wako Pure Chemical Corporation (Osaka, Japan), respectively. Toluene-d_8 standard stock solution (Kanto Chemical Co., Inc) was used as the internal standard. Methanol for pesticide residue-PCB analysis and carbon disulfide for assessment of working environment were purchased from Fujifilm Wako Pure Chemical Corporation. Carbon disulfide, the extraction solvent for inter-laboratory validation study, was prepared in each laboratory. Carbon beads active sampling tube filled with two layers of 20–40 mesh carbon beads (front layer: 100 mg, back layer: 50 mg; Sibata Scientific Technology Ltd., Tokyo, Japan) was used as adsorbents.

Sample Stability Test Sample stability tests were conducted under two different conditions at Laboratory C (Table 2).

[Condition I]

A standard solution containing 3 µg of each substance was spiked into the sampling tubes using a microneedle syringe, followed by passing 1 L of indoor air into the tubes. Polyethylene caps were put on both ends of the sampling tube, which were subsequently sealed with parafilm. One tube was analyzed immediately after preparation to confirm recovery of each substance as a day 0 sample. Other tubes were wrapped in aluminum foil and stored in a sealed container at 5°C (cool storage) for 4 and 7 days. Thereafter, these tubes were analyzed and recoveries were confirmed on day 4 and 7. Sampling tubes that contained only 1 L of indoor air were prepared as operation blanks. These tubes were also stored and analyzed at the same conditions as the spiked samples.

[Condition II]

A standard solution containing 1 µg of each substance was spiked into the sampling tubes using a microneedle syringe, followed by passing 1 L of indoor air into the tubes (*n*=3). Polyethylene caps were put on both ends of the sampling tube, which were then sealed with parafilm. The tubes were wrapped in aluminum foil and stored at 5°C for 6 days. Subsequently, the samples were analyzed to confirm recovery and repeatability (RSD$_r$). Sampling tube containing only 1 L of indoor air was analyzed as the blank.

Table 1. Present and Draft Guideline Values for the Target Substances

Substances	Guideline Value (µg/m³)	
	Present	Draft
Toluene	260	-
Xylene	200	-
Ethylbenzene	3,800	-
Styrene	220	-
p-Dichlorobenzene	240	-
Tetradecane	330	-
2-Ethyl-1-hexanol	-	130
2,2,4-Trimethyl-1,3-pentandiol monoisobutyrate (TPMI)	-	240
2,2,4-Trimethyl-1,3-pentandiol diisobutyrate (TPDI)	-	100

-: Undefined

Table 2. GC-MS Analysis Conditions in Each Laboratory

	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
Gas chromatograph	Shimadzu GC-2010	Agilent Technologies GC7890A	Shimadzu GC-2010 Plus	Agilent Technologies GC7890A	Agilent Technologies GC7890A
Column	DB-1 (Agilent) 0.25 mm i.d.×60 m, 1 µm	VF-1ms (Agilent) 0.25 mm i.d.×60 m, 1 µm	Rtx-1 (RESTEK) 0.32 mm i.d.×60 m, 1 µm	InertCap 1 (GL Sciences) 0.25 mm i.d.×60 m,1.5 µm	Rtx-1 (RESTEK) 0.25 mm i.d.×30 m, 1 µm
Injection volume	1 µL	1 µL	1 µL	1 µL	1 µL
Inlet mode	Split (1:10)	Split (1:50)	Split (1:5)	Splitless	Split (1:10)
Oven temperature	40°C (5 min)-10°C/min-270°C (4 min)	35°C (1 min)-1.5°C/min-70°C-3°C/min-100°C-8°C /min-315°C (1 min)	40°C -5°C/min-280°C (4 min)	45°C (5 min)-10°C/min -300°C (10 min)	40°C (10 min)-5°C/min-240°C -20°C /min-270°C (3 min)
Mass spectral detector	Shimadzu QP-2010 Ultra	Agilent Technologies MSD5975C	Shimadzu QP-2010 Ultra	Agilent Technologies MSD5977A	Agilent Technologies MSD5975C
Interface temperature	250°C	315°C	250°C	280°C	Not Reported
Ion-source temperature	200°C	230°C	200°C	250°C	Not Reported
Quantitative ion / Qualifying ion (*m/z*)					
Toluene	91 / 92	91 / 92	91 / 92, 65	91 / 92	91 / 92
Xylene	91 / 106	91 / 106	91 / 106, 105	91 / 106, 57	91 / 106
Ethylbenzene	91 / 106	91 / 106	91 / 106, 65	91 / 106	91 / 106
Styrene	104 / 105	104 / 103	104 / 78, 51	104 / 103	104 / 103
p-Dichlorobenzene	146 / 148	146 / 148	146 / 148, 111	146 / 148	146 / 148
Tetradecane	57 / 71	43 / 57	57 / 71, 43	43 / 57	57 / 71
2-Ethyl-1-hexanol	70 / 57	57 / 43	57 / 41, 43	57 / 83, 70	57 / 41
TPMI	71 / 89	71 / 43	71 / 43, 56	71 / 89	71 / 43
TPDI	71 / 43	71 / 43	71 / 43, 41	71 / 43	71 / 43
Toluene-d_8	98 / 100	98 / 100	98 / 100, 70	98 / No settings	98 / 100

Table 3. Extraction Conditions in Each Laboratory

	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
Solvent volume (mL)	1	2	2	2	2
Shake (min)	60	90	60	1	*
Stand (min)	-	-	-	120	
Centrifugation	-	-	3,000 rpm, 5 min	3,000 rpm, 10 min	-
Filtration	Syringe filter	-	-	-	Syringe filter
Internal standard concentration (µg/mL)	1	2	1	2.5	1

*: Stand 120 min → shake 1 min → stand 5 min → shake 1 min
-: Not performed

Table 4. Results of Sample Stability Test

Substances	Condition I (3 µg addition) Stored at 5°C, 7 days (n=1)			Condition II (1 µg addition) Stored at 5°C, 6 days (n=3)	
	Recovery (%)			Recovery (Average, %)	RSD_r (%)
	0 day	4 days	7 days		
Toluene	97.5	102	110	97.1	1.2
Xylene	96.1	99.5	108	95.0	0.79
Ethylbenzene	97.7	102	110	96.3	0.72
Styrene	83.5	84.2	88.1	81.8	0.84
p-Dichlorobenzene	91.5	96.1	104	88.9	0.60
Tetradecane	97.2	98.8	105	93.2	0.69
2-Ethyl-1-hexanol	94.6	97.6	102	90.1	2.7
TPMI	88.2	94.3	95.8	79.7	5.3
TPDI	81.7	89.8	92.4	78.4	4.9

RSD_r: Repeatability

Sample Preparation for Distribution   The additional amount in this validation study was set at 1 µg based on the new guideline values revised in 2019.[6] Xylene had the lowest guideline value among the target substances (200 µg/m³). Moreover, using the current SE method, 144 L of indoor air was collected (100 mL/min for 24 h) to analyze chemical substances, and approximately 30 µg of xylene was present in the sampling tube after aspirating 144 L air. Therefore, we set the additional amount to 1 µg for each substance, one-tenth of 30 µg after considering xylene isomers (*o*-, *m*-, and *p*-). To prepare distribution tubes, the standard solution containing 1 µg of each substance was spiked into the sampling tubes using a microneedle syringe and then 1 L of indoor air was passed through the tubes. After preparation of the distribution tubes, five tubes were analyzed immediately to confirm homogeneity. A blank, with both ends of the tube cut was also prepared for distribution. GC-MS and solvent extraction conditions are shown in Tables 2 and 3 (Laboratory A).

Inter-laboratory Validation Study   Five laboratories participated in this inter-laboratory validation study: Hokkaido Institute of Public Health (Sapporo, Hokkaido), Tokyo Metropolitan Institute of Public Health (Shinjuku-ku, Tokyo), Yokohama City Institute of Public Health (Yokohama, Kanagawa), Chiba Prefectural Institute of Public Health (Chiba, Chiba), and Nagoya City Public Health Research Institute (Nagoya, Aichi), which were randomly assigned as Laboratory A–E.

For the validation study, the accuracy, repeatability (RSD_r), and reproducibility (RSD_R) were evaluated based on the "guidelines for the validation of the testing method for drinking water" established by the MHLW.[12] Briefly, five spiked sampling tubes and a blank were delivered to each laboratory in cool storage. All test was performed within a week after sample preparation. Two layers of adsorbents in the sampling tubes were extracted together. The internal standard method was used for quantification with the distributed toluene-d_8 internal standard stock solution (1,000 µg/mL, methanol solution). For plotting calibration curves, the distributed standard stock solution (500 µg/mL, methanol solution) was diluted with carbon disulfide to a calibration range set at each laboratory. The calibration curve contained more than 5 points excluding the origin. Three peaks for xylene and two peaks for TPMI were observed on the chromatograph owing to the presence of isomers of xylene and TPMI. Therefore, the quantitative values were calculated by summing these peak areas. Other analytical conditions were set according to the standard method at each laboratory. GC-MS analysis and solvent extraction conditions are shown in Tables 2 and 3.

Calculation of Quantitative Value   Quantitative value of each substance was calculated by subtracting the value of the blank from the spiked (distribution) sample and used for calculation of accuracy, RSD_r, and RSD_R. The criteria ranges of the guideline values were accuracy of 70.0%–130%, RSD_r ≤20%, and RSD_R ≤25%.[12]

RESULTS AND DISCUSSION

Sample Stability Test   The change in recovery with time (day 0, 4, and 7) [condition I] and repeatability (RSD_r) after 6 days [condition II] for the spiked sample solutions were investigated. Table 4 shows the recovery of the five sampling tubes for condition I and the average recovery and RSD_r for condition II. Under condition I, recovery was not significantly different during the 7 days at 5°C. Additionally, under condition II, average recovery and RSD_r of all substances were above 70% and below 20%, respectively. These results indicated that recovery did not change with time and each substance on the

sampling tubes can be stably stored for about 1 week at 5°C. Therefore, the distribution tubes were stored in cool storage and all tests for inter-laboratory validation were performed within a week after the sampling tubes were prepared.

Homogeneity Test for Distribution Samples Before the spiked sampling tubes were distributed for inter-laboratory validation, five sampling tubes were randomly selected from 30 spiked sampling tubes to evaluate the recovery of all substances. Table 5 shows the average recovery and RSD_r of the five sampling tubes. The recoveries were over 70.0% for all substances, except styrene, and RSD_r was less than 10% for all target substances. Because the recovery of styrene can be low depending on the humidity and extraction time,[13] styrene-d_8 can be used as a surrogate to correct the recovery of styrene in inter-laboratory validation tests if necessary.[14] Although the recovery of styrene in this study was slightly low, its RSD_r was good at 3.9%. Homogeneity of the substances was almost confirmed by these results. Therefore, inter-laboratory validation test was conducted with these spiked sampling tubes.

Method Validation The concentration range of the calibration curves was different for each laboratory, but coefficient of determination values (R^2) were between 0.994 and 1.000, suggesting a good linearity at all laboratories. The accuracy and RSD_r evaluated from the quantitative value at each laboratory satisfied the criteria range with 71.9%–121% and 0.78%–9.6%, respectively, except in two cases (Table 6). The chromatogram peaks of the calibration samples showed low intensity for higher boiling point substances, including tetradecane, in Laboratory E compared with other laboratories (data not shown). These results suggest that a relatively high recovery of tetradecane was obtained. Therefore, it is essential to confirm the peaks in chromatogram of calibration samples in advance (i.e., balance of intensity, separation, retention time, and so on) and to maintain appropriate apparatus conditions for the analysis. Given the improvement in recovery when styrene-d_8 was used in Laboratory A (data not shown), it is important to correct quantification value, if necessary. Therefore, correction by using a surrogate is considered an effective approach, especially when recovery is low on prior confirmation, as observed in Laboratory C. Meanwhile, even including the two points, the average of accuracy and RSD_R among five laboratories were 75.4%–115% and 3.6%–21%, respectively, satisfying the criteria range.

To summarize, we performed an inter-laboratory validation study in five laboratories to confirm whether the current analytical method of VOCs in indoor air using solvent extraction is adaptable for the guideline values established after 2001. Satisfactory results were obtained even for additions of less than one-tenth of all the guideline values for VOCs, indicating that our analytical test method could be an efficient standard test method.

Conflict of interest The authors declare no conflict of interest.

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Table 5. Results of Homogeneity Test (*n*=5)

Substances	Recovery (Average, %)	RSD_r (%)
Toluene	90.0	2.7
Xylene	90.5	3.5
Ethylbenzene	94.1	3.2
Styrene	64.4	3.9
p-Dichlorobenzene	81.4	4.9
Tetradecane	97.6	4.7
2-Ethyl-1-hexanol	78.2	4.7
TPMI	93.0	7.1
TPDI	99.9	3.5

RSD_r: Repeatability

Table 6. Results of Inter-laboratory Validation Test

Substances	Accuracy (%)					RSD_r (%)					Accuracy (Average, %)	RSD_R (%)
	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E		
Toluene	94.4	99.0	91.3	99.8	96.8	4.3	2.5	3.6	3.2	1.3	96.3	3.6
Xylene	97.0	118	89.3	102	93.3	4.2	2.0	3.9	3.6	1.1	99.8	11
Ethylbenzene	100	106	91.1	103	97.4	4.3	4.0	3.9	3.6	0.78	99.6	5.8
Styrene	73.3	86.5	69.1	76.2	71.9	3.7	2.4	4.8	4.5	4.9	75.4	8.9
p-Dichlorobenzene	87.9	109	85.5	96.6	90.1	4.3	2.5	4.2	4.3	1.3	93.9	10
Tetradecane	108	118	89.9	106	154	4.0	3.2	5.4	5.7	9.6	115	21
2-Ethyl-1-hexanol	91.4	97.0	84.9	109	121	5.1	3.7	6.3	4.1	4.5	101	14
TPMI	104	98.6	85.6	104	117	7.1	6.7	7.0	9.6	2.3	102	11
TPDI	111	109	90.9	108	102	4.9	1.8	8.7	7.7	1.7	104	7.9

Additional amount: 1 µg (xylene: 3 µg)
RSD_r: Repeatability
RSD_R: Reproducibility

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