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Measurement Uncertainty of Nicotine in Environmental Tobacco Smoke (ETS)

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Nicotine is the main component of environmental tobacco smoke, and its presence in indoor air is widely used as a secondhand-smoke indicator. Environmental tobacco smoke is a major source of indoor air pollution, but sufficient investigation of the uncertainty of its measurement, which mirrors the reliability of nicotine measurement, has not been performed. We calculated the uncertainty of measurement of indoor air nicotine concentration at low, medium, and high concentrations of 11.3798, 10.1977, 98.3768 μg/m^3, respectively, and we employed the Guide to the Expression of Uncertainty in Measurements (GUM), proposed by the International Organization for Standardization (ISO). The factors considered in determining the uncertainty were uncertainty of the calibration curve (calibration curve and repeated measurements), desorption efficiency, extraction volume, and sampling airflow (accuracy and acceptable limits of flowmeter). The measurement uncertainty was highest at low concentrations; the expanded measurement uncertainty is 0.9435 μg/m^3 and is represented as a relative uncertainty of 63.38%. At medium and high (concentrations, the relative uncertainty was 13.1% and 9.1%, respectively. The uncertainty of the calibration curve was largest for low indoor nicotine concentrations. To increase reliability of measurement in assessing the effect of secondhand smoke, measures such as increasing the sample injection rate (1 μL or more), increasing sampling volume to increase collected nicotine, and using gas chromatography-mass spectrometry (GC/MS) or GC/MS/MS, which has a lower quantitation threshold, rather than gas chromatography with nitrogen phosphorous detector, should be considered.

Key Words : Nicotine, Uncertainty, Guide to the expression of uncertainty in measurements, Environmental tobacco smoke, Indoor air

Introduction

Environmental tobacco smoke (ETS) is the exhaust released on burning tobacco, and it often leads to passive smoking. Substances found in tobacco, such as nicotine or 3-ethenylpyridine have been used as indicators for ETS.[1-3] Among them, nicotine, is the main ingredient found in ETS, and because of its high specificity, it has been widely used as an indicator of ETS. ETS is classified as a Group A carcinogen, and in many epidemiological and risk assessment studies, exposure to ETS has been reported to increase the risk of respiratory and cardiopulmonary diseases, and lung cancer[4-6] Various studies of indoor ETS have been conducted in Korea and in other countries, to study the health effects of tobacco smoke and to set guidelines for its regulation. However, factors that lead to measurement uncertainty such as extraction volume, errors during instrument measurement and analysis, and the sensitivity of the instrument, limit the reliability of the measurements of nicotine as an indicator of secondhand smoke. The determination of measurement uncertainty is required by the International Organization of Standards (ISO) to express the reliability of the measured data and is used as a benchmark for the quality of the testing institution. The uncertainty of measurement is defined as the parameter that represents the dispersion of measurement results of a logically estimated measured quantity, based on the Guide to the Expression of Uncertainty in Measurements (GUM), issued by the ISO.[7] The nicotine concentration in indoor air can range from several micrograms per cubic meter to tens of micrograms per cubic meter. These are approximate or estimated values, and can only be complete when the uncertainty of measurement is indicated. Therefore, there is a need to evaluate the measurement uncertainty, which can indicate the reliability of the measured data. In this study, we determined the measurement uncertainty according to the GUM issued by the ISO. To do this effectively, we classified the indoor concentrations of nicotine into low, medium, and high categories, and calculated the uncertainty of measurement for each category.

Experimental Section

Reagents and Materials. The internal standards used for the quantification of nicotine were 99% nicotine solution (Sigma-Aldrich, USA) and 98% quinoline (Sigma-Aldrich, USA). The extraction solvent was 99% ethyl acetate containing 0.01% triethylamine (Junsei, Japan). Samples of indoor air were collected using the XAD-4 sorbent tube (SKC Inc. USA) connected to a sampling pump (SHIBATA, JAPAN) and set at a flow rate of 1 L/min for 3 h. The collected samples

Table 1. Analytical conditions

GC	Conditions
Model	HP 6890
Detector Temp.	280 °C
Injector Temp.	250 °C
Column	DB-5 (30 m × 0.25 mm I.D. × 0.25 μm film thickness)
Oven	60 °C (1 min) → (20 °C/min) → 250 °C (2 min)
Carrier Gas	N2, 1.0 mL/min
Split ratio	Splitless
Injection Volume	1 μL

were classified as low, medium, and high nicotine-concentration samples.

Instruments and Conditions for Analysis. The concentration of nicotine was analyzed using a gas chromatograph equipped with a nitrogen phosphorous detector (GC/NPD, 6890GC, Agilent), and the analysis conditions are listed on Table 1.

Washing and Extraction of Sample. All glassware used for the extraction and analysis of the samples were washed with the extraction solvent, and dried for 30 min at 60 °C, in an environment that is free of nicotine.

For extraction, the material found in the interior and posterior part of the sorbent tube were collected in a 2 mL vial, and were extracted ultrasonically using 1 mL of the extraction solvent containing 6 μg/mL of 98% quinoline (Sigma-Aldrich, USA), which is the internal standard.

Generation of the Calibration Curve. The concentrations of the nicotine standard samples used for the calibration curve were set at 0.1, 0.2, 1, 2, 10, and 20 mg/L, considering the concentration range in indoor air. Each sample was measured three times. The internal standard was 6 μg/mL of quinoline dissolved in the extraction solvent. The calibration curve was generated using the ratio of the peak area of the internal standard to the peak area of the nicotine standard.

Accuracy and Desorption Efficiency. Accuracy was measured by repeating thrice the analysis of the nicotine standard samples. Desorption efficiency was measured using the nicotine standard samples of concentration 0.2, 2, and 10 mg/L. The samples were added to a sorbent tube and were pre-treated using the standard procedure used for other samples. Each sample was measured seven times.

Model Equation for the Measurement Uncertainty of Nicotine. For the measured nicotine quantity (Y), the estimate (y) can be calculated based on the input $x_1, x_2, x_3, x_4 \ldots x_n$, represented by the following model equation.

$$y = f(x_1, x_2, x_3, x_4 \ldots x_n)$$

The effective degree of freedom was calculated using the Welch-Satterthwaite Eq. (1). The combined standard uncertainty was calculated using Eq. (2). By employing the coefficients of the partial differential Eq. (2), the expanded uncertainty (U) was calculated using Eq. (3).

The measurement uncertainty of nicotine concentration in the indoor air was determined at a confidence level of 95% by applying a coverage factor of ($K = 2$).

$$v_{eff} = \frac{u_e^4(y)}{\sum_{n=1}^{i} \frac{u_i^4(y)}{v_i}} \tag{1}$$

$$u_e(y) = \left[\left(\frac{\partial f}{\partial x_1}\right)^2 \times u(x_1)^2 + \left(\frac{\partial f}{\partial x_2}\right)^2 \times u(x_2)^2 \ldots \left(\frac{\partial f}{\partial x_n}\right)^2 \times u(x_n)^2\right] \tag{2}$$

$$U = k \times u_c \tag{3}$$

Based on the model Eq. (1), the concentration of nicotine in indoor air can be expressed as in Eq. (4). The measurement uncertainty for nicotine was determined by considering four factors: measurement from the instrument (C_o, mg/L), sample extraction volume (V_e, mL), sampling airflow volume through the suction pump (Q, L), and desorption efficiency (W_{DE}, %).

$$C_{nicotine} = \frac{C_o \times V_e}{Q \times \frac{W_{DE}}{100}} \times 1{,}000 \tag{4}$$

where

$C_{nicotine}$: concentration of nicotine in air, ug/m^3
C_o: concentration of nicotine measured at insrument, mg/L
V_e: extraction volume, mL
Q: air volume passed on adsoption tube, L
W_{DE}: desorption efficiency, %
1,000: conversion factor mg/L to ug/m^3

Results and Discussions

Measurement Uncertainty of Extraction Volume (uV_e). The uncertainty in the measurement of 1 mL of extraction volume (uV_e) was calculated as shown in Eq. (5). The error introduced due to the repeated use of the pipette was ignored, and only the certified calibration uncertainty was considered. The expanded measurement uncertainty of the calibration for the 1 mL pipette (U) was 0.029 mL. When divided by the coverage factor ($k = 2$), the uncertainty of extraction volume (uV_e) was 0.0145 mL. The effective degree of freedom was 200 at a confidence level of 95%.

$$uV_e = \frac{U}{k} \tag{5}$$

Measurement Uncertainty of Sampling Volume ($u_c(Q)$). The uncertainty of the sampling volume ($u_c(Q)$) was derived from the uncertainty of sampling flow volume of the sampling pump shown in Eq. (6) ($u(Q_{var})$) and the acceptable accuracy of the flowmeter ($u(Q_{pre})$) shown in Eq. (7).

The accuracy of the sampling volume and the accuracy of the flowmeter were set at 5%, which is the maximum acceptable error of the flowmeter. The sampling volume was 180 L at a flow rate of 1 L/min for using a multiplication factor

of 5% and assuming B-type rectangular distribution. The uncertainty of changes in the flow rate ($u(Q_{var})$) and the volume accuracy ($u(Q_{pre})$) were both 5.20 L. The effective degree of freedom was 200, at a confidence level of 95%. Therefore, the combined standard uncertainty of sampling volume ($u_c(Q)$) was 7.35 L, based on Eq. (8), and the effective degree of freedom was 400.

$$u(Q_{var}) = \frac{Total\ sampling\ volume(180\ L) \times T_v\ of\ pump(5\%)}{\sqrt{3}} \quad (6)$$

$$u(Q_{pre}) = \frac{Total\ sampling\ volume(180\ L) \times T_p\ of\ pump(5\%)}{\sqrt{3}} \quad (7)$$

$$u_c(Q) = \sqrt{(u(Q_{var}))^2 + (u(Q_{pre}))^2} \quad (8)$$

Measurement Uncertainty of the Sample Desorption Efficiency ($u(W_{DE})$). Measurement uncertainty of the sample desorption efficiency ($u(W_{DE})$) was analyzed by repeating the measurement seven times (n = 7), for each concentration group: low concentration (0.2 mg/L), medium concentration (2 mg/L), and high concentration (10 mg/L). The average desorption efficiency of the low concentration (0.2 mg/L), medium concentration (2 mg/L), and high concentration (10 mg/L) samples were 88.24%, 101.29%, and 98.37%, respectively, and the average desorption efficiency of all the groups was 95.97%. The pooled standard deviation of 5.659%, calculated using the variance at each concentration ($S^2_{W_{DE\ low}}$, $S^2_{W_{DE\ medium}}$ and $S^2_{W_{DE\ high}}$), was entered into equation (9), divided by the total number of measurements (N = 21), to calculate the measurement uncertainty of the sample desorption efficiency, which was 1.235% (Table 2). The effective degree of freedom was 18.

$$u(W_{DE}) = \frac{\left[\frac{(n-1)S^2_{W_{DE\ low}} + (n-1)S^2_{W_{DE\ medium}} + (n-1)S^2_{W_{DE\ high}}}{(N-3)}\right]^{\frac{1}{2}}}{\sqrt{N}} \quad (9)$$

Measurement Uncertainty of Analyzed Nicotine Concentration ($u_c(C_o)$). The measurement uncertainty of the concentration of nicotine determined using the instruments ($u(C_o)$), was derived using the uncertainty of measurement of repeated measurements of the standard ($u(C_{rep})$) and the uncertainty of measurement from the calibration curve ($u(C_{cal})$).

Table 2. Desorption efficiency data and standard uncertainty

Repeat number	Desorption efficiency (%)		
	0.20 mg/L	2.0 mg/L	10 mg/L
1	96.73	95.50	98.04
2	82.78	97.02	101.45
3	81.64	101.96	98.74
4	84.46	107.70	97.62
5	96.71	106.91	92.61
6	79.28	96.89	101.85
7	96.10	103.05	98.31
Average	88.24	101.29	98.37
Standard deviation	7.89	4.96	3.04
Variance	62.27	24.56	9.27
Pooled standard deviation		5.659	
Standard uncertainty, $u(W_{DE})$		1.235	
Degree of freedom		18	

The measurement uncertainty of repeated measurements of the standard ($u(C_{rep})$) was calculated using the same method as the one used for calculating the measurement uncertainty of the desorption efficiency ($u(W_{DE})$); however, a minor difference in the latter was in the value of N (N = 18). The measurement uncertainty of repeated measurements was 0.019 mg/L, and the effective degree of freedom was 12 (Table 3).

The measurement uncertainty of the calibration curve ($u(C_{cal})$) was calculated by measuring the six standard solutions (0.1, 0.2, 1, 2, 10, and 20 mg/L), three time each. The standard error in the calculation of the residuals, slope, and intercept, needed for the calculation of the uncertainty of the calibration curve, was determined using regression analysis. The calibration curve was calculated using the ratio of the area of nicotine standards and the internal standards (Table 4).

The nicotine sample concentration, obtained from the

Table 3. Standard uncertainty of repeated measurement

Repeat number	Concentration of standard solution (mg/L)					
	0.10	0.20	10	2.00	10.00	20.00
1	0.15	0.22	0.93	1.82	9.55	19.07
2	0.14	0.22	0.92	1.86	9.47	19.08
3	0.13	0.22	0.94	1.88	9.70	19.29
Average	0.14	0.22	0.93	1.85	9.58	19.15
Accuracy (%)	141.73	110.25	93.09	92.55	95.76	95.73
Standard deviation (mg/L)	0.005975	0.002222	0.009116	0.030222	0.116727	0.123969
Variance	0.000036	0.000005	0.000083	0.000913	0.013625	0.015368
Pooled standard deviation			0.082			
Standard uncertainty, $u(C_{rep})$			0.019			
Degree of freedom			12			

Table 4. Calibration curve data and standard uncertainty

Concentration (mg/L)	As/Ai	Sum of square x
0.1	0.0012	29.7025
0.1	0.0011	29.7025
0.1	0.0010	29.7025
0.2	0.0023	28.6225
0.2	0.0023	28.6225
0.2	0.0022	28.6225
1	0.0125	20.7025
1	0.0124	20.7025
1	0.0126	20.7025
2	0.0254	12.6025
2	0.0260	12.6025
2	0.0262	12.6025
10	0.1373	19.8025
10	0.1361	19.8025
10	0.1395	19.8025
20	0.2750	208.8025
20	0.2751	208.8025
20	0.2782	208.8025
Average = 5.55		Sxx = 960.71

Calibration uncertainty factor of ISO GUM	
s (Standard error of the residuals)	0.001
P (Number of measurements to determine concentration)	1
n (Number of measurements for the calibration)	18
S_{xx} (Sum of squares, x)	960.71
Co (Calculated value of sample)	0.238
$\overline{C}$ (Mean value of the different calibration standards)	5.55
B1 (Slope)	0.0138
Standard uncertainty(uC_{cal})	0.078
Degree of freedom	16

As/Ai: Area of nicotine standard/Area of internal standard

$$u(C_{cal}) = \frac{s}{B1}\sqrt{\frac{1}{P}+\frac{1}{n}+\frac{C_o-\overline{C}}{S_{xx}}}$$

calibration curve, was analyzed at low, medium, and high concentration. The calculated value of low concentration (C_o) was 0.238 mg/L, and those of the medium and high concentrations were 1.762 and 16.994 mg/L, respectively.

Expanded Measurement Uncertainty for Nicotine Concentration ($U(C_{nicotine})$). The expanded measurement uncertainty for nicotine concentration can be calculated either by dividing each uncertainty of measurement by the sampled volume (relative measurement uncertainty), or by using the partial differential values derived from the partial differential of the model equation. The partial differential is called the sensitivity coefficient, and in this study, we used the sensitivity coefficient and individual measurement uncertainty to derive the expanded measurement uncertainty of the nicotine concentration. The concentration of nicotine in indoor air (C_o) measured by the instrument was categorized into low concentration (0.238 mg/L), medium concentration (1.762 mg/L), and high concentration (16.994 mg/L). Table 5 lists the factors used in the measurement of the low-concentration nicotine (0.238 mg/L) sample.

Table 5. Result of standard uncertainty

Symbol	Unit	Value	SU Source	SU	DF	SC
C_o	mg/L	0.238	$u_c(C_o)$	0.080 8	18	5.789
			$u(C_{cal})$	0.078 4	16	
			$u(C_{rep})$	0.019 3	12	
V_e	mL	1.000	$u(V_e)$	0.014 5	200	1.380
W_{DE}	%	95.97	$u(W_{DE})$	1.235 1	18	-0.014
			$u(W_{DE\,low})$	2.982 6	6	
			$u(W_{DE\,medium})$	1.873 0	6	
			$u(W_{DE\,high})$	1.150 9	6	
Q	L	180	$u_c(Q)$	7.348 5	400	-0.008
			$u(Q_{var})$	5.196 2	200	
			$u(Q_{pre})$	5.196 2	200	

DF: Degree of freedom, SD: Sensitive Coefficient, SU: Standard uncertainty

The value measured by the instrument was converted to get the concentration of nicotine in indoor air (ug/m^3) using 1 mL extraction volume, 180 L sampling volume, and 95.96% average extraction efficiency. The converted value was 1.3798 μg/m^3, and the effective degree of freedom was 17. Using the effective degree of freedom, the coverage factor (k) calculated based on the t-distribution at 95% confidence level was found to be 2.12. The combined standard measurement uncertainty for the nicotine concentration (was 0.4717 μg/m^3, and the expanded measurement uncertainty calculated using the coverage factor (k = 2) and combined standard measurement uncertainty was 0.9435 μg/m^3 (k = 2, 95% confidence level).

Using the same method, the expanded measurement uncertainty of medium concentration of nicotine (10.1977 μg/m^3) in indoor air was found to be 1.3324 μg/m^3 (k = 2, 95% confidence level), and the expanded measurement uncertainty at high concentration of nicotine (98.38 μg/m^3) in indoor air was found to be 8.9492 μg/m^3 (k = 2, 95% confidence level). The relative measurement uncertainty, calculated by dividing the measurement uncertainty by the measured quantity (1.38 μg/m^3), was 68.4% for the low-nicotine-concentration sample. The relative measurement uncertainty calculated using the same method for medium and high concentrations of nicotine were 13.1% and 9.1%, respectively.

Conclusion

The measurement of the concentration of nicotine in indoor air includes uncertainties stemming from the errors occurring in sampling and analysis. By evaluating the measurement uncertainty factors, the reliability of the results and the proficiency of the analytical technology used for the measurements can be determined.

In this study, we classified the concentration of nicotine in indoor air into low, medium, and high categories, and using the concept of uncertainty of measurement, we determined the range of measurement uncertainty for each category. In particular, our study is significant because we introduced the concept of uncertainty of measurement to determine the

concentration of nicotine, which is used as an indicator of secondhand smoke.

The measurement uncertainty was derived using the Guide to the Expression of Uncertainty in Measurements, and the four factors considered were uncertainty of the calibration curve (calibration curve and repeated measurements), desorption efficiency, extraction volume, sampling air flow (accuracy and acceptable limits of the flowmeter).

The highest expanded measurement uncertainty was 0.9435 $\mu g/m^3$ (63.38%) for the low concentration (1.3798 $\mu g/m^3$) of nicotine. This is because at low nicotine concentrations, the uncertainty of measurement of the calibration curve during instrumental analysis is high. At medium and high concentrations, the relative measurement uncertainties were 13.1% and 9.1%, respectively. In summary, to increase reliability of measurement for low concentrations of nicotine, measures such as fast sample injection rates (1 μL or more), large sampling volume to increase nicotine collection, and instruments that have a lower quantification threshold such as gas chromatography-mass spectrometry (GC/MS) or GC/MS/MS, rather than gas chromatography with nitrogen phosphorous detector (GC/NPD), should be considered.

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