Determination of Formaldehyde in Aqueous Samples with a Miniaturized Extraction Capillary Coupled to High-Performance Liquid Chromatography

Ikuko UETA,^{*,†} Suguru MOCHIZUKI,^{*} Susumu KAWAKUBO,^{*} Tetsuo KUWABARA,^{*} and Yoshihiro SAITO^{**,†}

^{*}Department of Applied Chemistry, University of Yamanashi, 4-3-11 Takeda, Kofu 400-8511, Japan

^{**}Department of Environmental and Life Sciences, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempakuchō, Toyohashi 441-8580, Japan

A novel analytical method for determining formaldehyde (FA) in aqueous samples was developed with a miniaturized extraction capillary in high-performance liquid chromatography (HPLC). The extraction capillary was prepared by packing silica gel particles in a stainless steel capillary of 0.8 mm i.d. and 1.6 mm o.d. A derivatization reagent of 2,4-dinitrophenylhydrazine (DNPH) was impregnated on the silica gel particles by pumping a solution of DNPH to the capillary. Two extraction methods; dynamic extraction and purge-and-trap (PT) methods, were investigated for derivatizing and extracting FA from water samples onto the silica gel in the extraction capillary. The extraction capillary was directly connected to a six-port valve, and then desorption of the derivative from the capillary and injection to conventional HPLC system were simultaneously achieved with a flow of acetonitrile through the capillary. In the dynamic extraction, FA was determined with a simple sample preparation procedure, and the limit of detection (LOD) was 18 $\mu\text{g L}^{-1}$ at a sample volume of 20 mL, while several limitations were found in the method, such as bleeding of the derivatization reagent and the corresponding derivatized FA from the capillary during the sample load/extraction process. The sensitivity was significantly improved by introducing a PT technique, where the LOD was 6.9 $\mu\text{g L}^{-1}$ at a sample volume of 20 mL with a sampling time of approximately 20 min. With the PT method, successful recoveries of FA were confirmed for spiked tap water, river water and fruit juice samples.

Keywords Formaldehyde, derivatization, sample preparation, purge and trap, HPLC

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Introduction

Determination of volatile organic compounds (VOCs) in water samples has been regarded as an important technique to monitor VOCs levels in environmental samples and also to avoid adverse effects on human health. According to the International Agency for Research on Cancer (IARC), formaldehyde (FA) is classified as a carcinogen,¹ and the guideline value of FA in drinking water has been determined at 900 $\mu\text{g L}^{-1}$ by the World Health Organization (WHO).² The corresponding guideline value in Japan is published by the Ministry of Health, Labour and Welfare (MHLW) to be 80 $\mu\text{g L}^{-1}$ for tap water, because all the tap water is potable and therefore can be normally regarded as drinking water in Japan. For the determination of FA in aqueous samples, a derivatization reaction with *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine (PFBHA) and subsequent extraction into hexane followed by gas chromatographic (GC) analysis has been typically employed as the official method recommended by the United States Environmental Protection Agency (U.S. EPA).³ A sensitive detection of FA in aqueous

samples could be achieved in this analytical method, however; complicated multistep processes are needed along with careful operation by a skilled analyst for repeatable results.

A needle-type extraction device has been employed as the extraction tool of VOCs in gaseous samples because of its excellent sensitivity as well as simplified operation.⁴⁻⁶ Introducing the needle-type extraction device packed with an activated carbon-based extraction medium, very volatile organic compounds could be effectively extracted,^{7,8} although an appropriate derivatization process is needed for sensitive determination of FA in GC.^{9,10} The extraction needle was further applied to extracting VOCs in water samples with the purge-and-trap (PT) technique.^{11,12} A dynamic extraction of organic compounds from water samples was also investigated using a fiber-packed extraction needle as the sample preparation device.¹³

Recently, our research group developed a novel miniaturized extraction capillary for the determination of gaseous FA in high performance liquid chromatography (HPLC).¹⁴ The extraction capillary has silica gel particles in a stainless steel capillary of 0.8 mm i.d. and 1.6 mm o.d. Before collection of the gaseous sample, a solution of 2,4-dinitrophenylhydrazine (DNPH) was loaded into the capillary, and DNPH was impregnated into the packed silica gel particles. During the sample collection, FA

[†] To whom correspondence should be addressed.
E-mail: iueta@yamanashi.ac.jp

was reacted with DNPH and extracted onto the silica gel. The extraction capillary was then directly connected to a conventional six-port valve, and simultaneous desorption and sample loading was carried out for the analysis in HPLC. The developed capillary extraction method is based on the well-known derivatization reaction with DNPH; however, it shows several advantageous features over the conventional time-consuming procedure using solid-phase extraction (SPE) as the sample preparation step before the HPLC analysis.^{15,16}

In this study, FA in aqueous samples was determined using the miniaturized sample extraction device followed by the separation in HPLC. Two types of extraction methods; *i.e.* dynamic extraction and PT extraction, were investigated. The PT method showed sensitive determination of aqueous FA, and the quantitative recovery of FA was confirmed from tap water, river water and fruit juice samples. The PT method was also applied to the determination of FA in aqueous extracts of dried shiitake mushroom.

Experimental

Chemicals and materials

Formaldehyde-2,4-dinitrophenylhydrazine (FA-DNPH) and DNPH hydrochloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Acetonitrile (aldehyde analysis grade) was obtained from Kanto Chemical (Tokyo, Japan). Standard FA solution of the desired concentration was prepared from a 36% aqueous FA solution (Kanto Chemical). Derivatization reagent of DNPH solution was prepared every week.

Miniaturized extraction capillary

Porous silica particles (60/80 mesh, relative surface area $700 \text{ m}^2 \text{ g}^{-1}$; Shinwa Chemical Industries, Kyoto, Japan) were packed into a stainless steel capillary (0.8 mm i.d., 1.6 mm o.d., 35 mm length). The packing length of the silica particles were 30 and 10 mm for dynamic extraction and PT extraction, respectively. A small amount of Zylon filaments (Toyobo, Shiga, Japan) were also packed at each end of the packing section to maintain the packed silica gel particles.

The extraction capillary was thoroughly washed with acetonitrile before use. DNPH solution (1000 g L^{-1} in acetonitrile) of 100 μL was loaded onto the capillary, and DNPH was impregnated onto the packed silica gel particles. The capillary was then attached to a vacuum gas sampling device (Komyo Rikagaku Kogyo, Tokyo, Japan), and 100 mL of dried N_2 gas was passed through the capillary to dry the silica gel particles. The preconditioning step of the extraction capillary is illustrated in Fig. 1.

After the extraction and derivatization of FA, the capillary was connected to a six-port valve using a PEEK nut, where the tip of the capillary was connected to the valve and another side was connected to a syringe containing the desorption solvent (50 μL of acetonitrile). The desorption was made as follows: 25 μL of acetonitrile was loaded into the capillary and held for 3 min, then the remaining 25 μL of acetonitrile was additionally loaded. After loading the desorption solvent, the injection was made by switching the six-port valve. More than 90% of FA-DNPH was desorbed from the capillary in this desorption method. After the analytes had been desorbed, the extraction capillary was washed with acetonitrile, and the device was reused for another measurement.

Dynamic extraction

For dynamic extraction of aqueous FA, an aqueous sample of

Fig. 1 Illustration of (A) dynamic extraction and (B) PT methods using an extraction capillary.

10–40 mL was collected in a plastic syringe, and the syringe was installed to a syringe pump (Model 11 Syringe Pump, Harvard Apparatus, Holliston, MA). The DNPH impregnated extraction capillary was connected to the syringe using a PTFE tube. The sample solution was loaded into the extraction capillary at approximately 2 mL min^{-1} as illustrated in Fig. 1A.

PT extraction

Aqueous samples (20 mL) were prepared in a 50 mL glass vial, where two silicon septa were attached to the plastic cap of the vial. NaCl was added to the sample solution if necessary. The sample solution was ultrasonicated for 1 min, and then stirred for 5 min in a water bath to maintain a constant temperature. The PT temperature was investigated in the range of 20 to 50°C . The PT extraction was performed as shown in Fig. 1B, where two needles were inserted into the vial: one is connected to the extraction capillary and another is connected to N_2 gas bag to supply pure N_2 as the purge gas. A PTFE tube was used for connecting the extraction capillary and a blank needle. The extraction was made by a gas sampling pump (GSP-300FT-2, Gastec, Kanagawa, Japan), and the sampling speed of the headspace gas in the vial was set at 100 mL min^{-1} . The sampling volume was investigated between 100 to 2000 mL.

HPLC system

Chromatographic separation was performed using a Shimadzu LC-10AD pump (Shimadzu, Kyoto, Japan) and an SPD-20A UV-Vis detector (Shimadzu) using the detection wavelength at 360 nm. For the separation, an octadecylsilica analytical column (5 μm particle size, 4.6 mm diameter, 150 mm long; Shinwa Chemical Industries) was employed. A six-port valve (Rheodyne, Cotati, CA) was used as the injector, and a 50 μL sample loop was installed to ensure the injection was successful. The mobile phase was a mixture of acetonitrile (50%) and water (50%) and the flow rate was 1 mL min^{-1} . The chromatographic data were acquired using a C-R8A recorder (Shimadzu).

Results and Discussion

Dynamic extraction

First, an FA solution of $200 \mu\text{g L}^{-1}$ was pumped through the extraction capillary by changing the solution volume from 10 to 40 mL, and the resulting peak area was measured as illustrated

Fig. 2 Peak area of FA-DNPH at different sample loading volume in dynamic extraction method obtained with (a) constant FA concentration of $200 \mu\text{g L}^{-1}$, and (b) constant total FA amount ($4 \mu\text{g}$).

Fig. 3 Effect of sodium chloride concentration on PT extraction. Sample concentration; $80 \mu\text{g L}^{-1}$, purge temperature; 40°C , sampling volume of head space gas: 2000 mL.

in Fig. 2a. Ideally, an increasing peak area was expected with increasing the sample loading volume because DNPH and FA-DNPH are not highly water soluble; however, the peak area decreased with increasing the sample loading volume. A constant amount ($4 \mu\text{g}$) of FA was also loaded to the extraction capillary using a set of FA solutions having different concentrations (Fig. 2b), where the sample loading volume was changed on the basis of these sample concentrations to maintain a constant total amount of the loaded FA. In this case, a constant peak area should be obtained with increasing sample loading volume, although the peak area decreased with increasing the sample loading volume. These results suggested bleeding of DNPH and FA-DNPH from the extraction capillary during the above two sample loading processes. It seems that DNPH and FA-DNPH were slightly dissolved in water, and then they moved to the outlet side of the extraction capillary during the loading of the sample solution.

The dynamic extraction method was evaluated by passing 20 mL of sample solution. The relative standard deviation (RSD) of the peak area ($n = 6$) of the dynamic extraction with $200 \mu\text{g L}^{-1}$ of sample concentration was about 12%. A linear calibration was obtained from 8 to $1000 \mu\text{g L}^{-1}$ with a good coefficient ($r^2 > 0.99$). The standard deviation (SD, $n = 6$) of blank FA-DNPH ($8 \mu\text{g L}^{-1}$ FA sample) was 750, while the peak area was approximately 6000. The limit of detection (LOD) and limit of quantification (LOQ) of the dynamic extraction method were 18 and $39 \mu\text{g L}^{-1}$, respectively. The LOD and LOQ were determined from the SD of the FA peak area measured using $8 \mu\text{g L}^{-1}$ samples. In this evaluation, the LOD was estimated as follows: first the SD obtained with $8 \mu\text{g L}^{-1}$ sample was triplicated, and then divided by the slope of the calibration line; next $8 \mu\text{g L}^{-1}$ was added to the above calculation result. In the case of the LOQ, the same calculation was carried out except for that based on 10 SD.

In general, it can be said that the dynamic extraction method was a simple sample preparation method, however, the sensitivity was not enough to analyze typical environmental samples in this work. In addition, a limitation in the sensitivity was found

because of the bleeding of DNPH and FA-DNPH from the capillary during the sample loading process.

PT extraction

FA has high water solubility, therefore, as expected, a lower purge efficiency of FA from water samples was expected in a PT method. In order to obtain satisfactory sensitivity of FA in the PT method, a large volume sampling of headspace gas is needed. Therefore, the packing length of silica gel particles was determined to be 10 mm in the PT extraction to introduce a higher sampling speed. The maximum sampling speed for gaseous sample (head space gas) was more than 100 mL min^{-1} in this extraction capillary.

The sampling volume of headspace gas was investigated from 100 to 2000 mL using a standard aqueous sample of $80 \mu\text{g L}^{-1}$. Because the peak area of FA-DNPH linearly increased up to 2000 mL of sampling, the sampling volume was set at 2000 mL. Typically, purge efficiency of water soluble VOC increased with the addition of salt by the salting out effect. The effect of NaCl addition was investigated from 0 to 30% as illustrated in Fig. 3. The peak area of FA-DNPH increased with the NaCl concentration of 0 to 20%, and then it reached a plateau at a concentration of more than 25%. Based on this result, the concentration of NaCl was optimized at 25%.

The effect of purge temperature (temperature of sample solution during PT) was investigated in the range from 20 to 50°C . Because purge efficiency of FA increased with increasing purge temperature, a linear increase of the peak area of FA-DNPH was expected. However, the peak area decreased at the purge temperature of 50°C as plotted in Fig. 4, suggesting an effect of excess water vapor at a higher purge temperature. Hence, the purge temperature of 40°C was chosen as the optimum value.

A linear calibration was obtained in the sample concentration range between 3 to $10000 \mu\text{g L}^{-1}$. The LOD and LOQ were calculated in the same manner as described above. The LOD and LOQ at the optimized condition are tabulated in Table 1, indicating a sensitive detection of aqueous FA by the PT method

Fig. 4 Effect of purge temperature on PT extraction. Sample concentration; 80 $\mu\text{g L}^{-1}$, sodium chloride concentration; 25%.

Table 1 LOD and LOQ of FA by dynamic and PT method with the extraction capillary

	Dynamic	PT
LOD/ $\mu\text{g L}^{-1}$	18	6.9
LOQ/ $\mu\text{g L}^{-1}$	39	16

using the extraction capillary. The RSD of the peak area of FA-DNPH was 5.6% at the concentration of 80 $\mu\text{g L}^{-1}$ ($n = 6$). A typical extraction capillary could be reused more than 50 times without any significant decrease in its extraction and desorption performance. The reproducibility of the extraction capillary was evaluated for five different capillaries, and the RSD of peak area for 80 $\mu\text{g L}^{-1}$ of a standard sample was less than 9.5%.

The recoveries of FA from tap water (Yamanashi, Japan), river water (Fuefuki River, Yamanashi, Japan) and fruit juice samples were investigated as illustrated in Table 2. All the investigated juices were commercially available not-from-concentrate juice, and diluted with pure water to avoid bubble formation during the PT extraction. The dilution rate for apple, orange and grape juices were determined as 2, 4 and 10 times, respectively, based on a preliminary study. FA was not detected in non-spiked tap water, river water and apple juice samples, while FA was detected in orange and grape juice samples. Then, standard FA was spiked into these samples at concentrations of 40 and 80 $\mu\text{g L}^{-1}$. The recoveries were calculated by using a calibration curve prepared using standard samples. The recoveries of FA were within a range from 97 to 103%, showing a successful recovery from real environmental samples and juice samples. Because the PT method could allow for the extraction of only volatile compounds onto the extraction capillary, FA was quantitatively determined in the proposed method without any interference from other compounds in complex sample matrices.

Finally, the developed PT method was also applied to the determination of FA in aqueous extracts of dried shiitake mushroom. Three types of commercially available dried shiitake mushrooms (all the shiitake mushroom were from Japan, and made by different manufacturers) were immersed in 100 mL of

Table 2 Recoveries of FA from real samples

Sample	Add/ $\mu\text{g L}^{-1}$	Found/ $\mu\text{g L}^{-1}$	Recovery, %
Tap water	0	< LOD	—
	40	43	103
	80	84	103
River water	0	< LOD	—
	40	39	99
	80	81	101
Apple juice	0	< LOD	—
	40	39	98
	80	84	102
Orange juice	0	97	—
	40	136	99
	80	171	97
Grape juice	0	223	—
	40	262	100
	80	302	100

($n = 6$).

Table 3 Determination of FA in dried shiitake mushroom samples

Sample	Dry weight/mg	Found/ $\mu\text{g L}^{-1}$
A	2.9	569
B	2.4	618
C	6.5	440

($n = 3$).

pure water for 3 h, then the shiitake mushrooms were removed from the water. The obtained water samples were determined by the PT method as shown in Table 3. Higher FA concentrations were found in the shiitake mushroom extracts as previously reported elsewhere.¹⁷ The results indicated that the concentrations of FA did not depend on the weight of dried shiitake mushroom, and that it could be more related to the components included in the shiitake mushroom. Taking advantage of the wide linear range of the determination in the proposed technique, various types of complex sample mixtures including natural products could be analyzed without changing experimental conditions in the present method.

Conclusions

Aqueous FA was sensitively determined by the developed PT method using a silica gel particle-packed extraction capillary. The developed method allowed for rapid and simple sample preparation compared to traditional methods, and also provided satisfactory sensitivity. Good recoveries were obtained for the FA from spiked tap water and river water samples, and the method could selectively determine FA from complex sample matrices, such as fruit juices. Relatively higher concentrations of FA were also successfully determined from the water samples containing the extracts from dried shiitake mushroom by the proposed PT method.

The extraction capillary showed HPLC determination of FA in both gaseous and aqueous samples with a simple sample preparation method. The developed method could be further applied to the extraction of volatile aldehydes and ketones in

aqueous samples.¹⁴

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