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Determination of Formaldehyde in Water Samples by High-Performance Liquid Chromatography with Methyl Acetoacetate Derivatization

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Abstract
A high-performance liquid chromatography method with methyl acetoacetate derivatization via the Hantzsch reaction was developed for the analysis of formaldehyde (HCHO) in several water samples. Under optimized conditions, HCHO was detected within 4 min and was not affected by excessive derivatization reagents. The calibration curve constructed from the peak height of HCHO was linear, with a correlation coefficient of 0.9998. The relative standard deviation of the peak height from ten replicates was 0.29%. The detection and quantitative limits were 0.96 µg/L and 3.16 µg/L, respectively. A recovery test of HCHO was performed to compare the developed method with the official analysis method (DNPH method). The developed method was used to determine the HCHO levels in several water samples (tap water, river water, and waste water).

Keywords Formaldehyde · High-performance liquid chromatography · Hantzsch reaction · Derivatization · Methyl acetoacetate · Water samples

Formaldehyde (HCHO) is the simplest structural aldehyde contained in adhesive agents, paints, and preservatives. However, HCHO is a well-known carcinogen (Kerns et al. 1983), and may adversely affect the human body and ecosystems. For example, it can irritate mucous membranes, which can result in acute toxicity (Til et al. 1989; Rusch et al. 1983; Marks et al. 1980). In addition, severe irritation and inflammation can occur when the human skin and eyes come in contact with an aqueous solution of HCHO. Moreover, the LD_{50} value for algae is 0.3–22 mg/L, and plankton and other sea plant are susceptible to even trace amounts of HCHO, which may affect the ecosystem (Burridge et al. 1995). In light of these factors, the Ministry of Health, Labour and Welfare, Japan, has mandated that the tap water should not contain more than 0.08 mg/L of HCHO (Ministry of Health, Labour and Welfare 2015). For aquatic life, the level of HCHO has been set at 1.0 mg/L by the Ministry of the Environment, Japan (Ministry of the Environmental 2003).

Two analytical methods have been adopted as the official method for detecting HCHO in tap water. One is solvent-extraction gas-chromatography mass-spectrometry (SE-GC/MS). Because it is difficult to detect the simple HCHO molecule using liquid and gas chromatography, it is necessary to derivatize the HCHO in the sample (Szulejko and Kim 2015). In SE-GC/MS, *o*-(2,3,4,5.6-pentafluorobenzyl) hydroxylamine (PFBOA) (Cullere and Cacho 2004; Beranek and Kubatova 2008) is used as a HCHO derivatization reagent. However, the sample must be left to stand for 3 h during the derivatization procedure; therefore, it takes a long time to start the measurement. In addition, the derivatization operation is complicated because solvent extraction using hexane is necessary in this process. The second method is high-performance liquid chromatography (HPLC) with 2,4-dinitrophenylhydrazine (DNPH) derivatization and has been adopted as the new official analytical method since 2016. In this method, DNPH (Dong and Moldveanu 2004; Wang et al. 2012) is used as a HCHO derivatization reagent. The derivatization procedure is simple, and the time required is much shorter than that for SE-GC/MS. However, DNPH is extremely reactive and can react with aldehydes in air. This can decrease the reagent purity and increase the blank value of the measurement. Moreover, DNPH is a known mutagen and carcinogen.

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The Hantzsch reaction with acetylacetone is a HCHO derivatization reaction that differs from the aforementioned methods (Li et al. 2007, 2008; Guzman et al. 2018). In this reaction, aldehydes react with 1,3-dicarbonyl compounds such as 1,3-diketone or 2-ketoester, and finally generate 1,4-dihydropyridine in the presence of ammonia. This reaction is commonly used in the synthesis of pharmaceutical products (Bossert et al. 1981). Previously, we tried to analyze the HCHO levels in several water samples using the HPLC with acetylacetone derivatization method: while analysis of the HCHO levels in tap water gave satisfactory results, analysis of the HCHO levels in natural water samples such as river water was difficult using this method (Ishikawa et al. 2014).

In this study, we demonstrate the determination of HCHO levels in several water samples (tap water, river water, and waste water) by HPLC using methyl acetoacetate instead of acetylacetone as a derivatization reagent. First, we confirmed the stability of the HCHO-methyl acetoacetate derivative. Then, we compared the DNPH method, one of the current official HCHO analysis methods in Japan, with the proposed method, and confirmed the usefulness of this method. Finally, the proposed method was used to determine the levels of HCHO in several water samples.

Materials and Methods

The equipment consisted of a DG-1580-53 three-line degasser, a PU-1580 intelligent HPLC pump, a CO-1565 intelligent column oven, and an MD-1515 multi wavelength detector (JASCO Corporation, Tokyo, Japan). A model 7725 injection port (Rheodyne, Cotati, CA, USA) equipped with a 100 µL sample loop was used as a manual injection valve. All measurements with the HPLC system were performed using a Mightysil RP-18 GP column (4.6 mm i.d. × 150 mm, Kanto Chemical, Tokyo, Japan). ChromNAV software (JASCO Corporation, Tokyo, Japan) was used for data acquisition and data handling.

Special grade acetonitrile and methanol (Wako Pure Chemical, Osaka, Japan) were used in the mobile phase. Formaldehyde standard solution (1000 mg/L in methanol, for chemical analysis) was obtained from Kanto Chemical (Tokyo, Japan). Each standard solution was prepared by diluting with the mobile phase. Ammonium acetate (Wako Pure Chemical, special grade) and methyl acetoacetate (first grade, Tokyo Chemical Industry, Tokyo, Japan) were used to prepare the HCHO derivatization reagent.

When comparing with the official analysis method, the aforementioned acetonitrile was purchased from Wako Pure Chemical and used in the mobile phase. Phosphoric acid (Wako Pure Chemical, for boron analysis) and 2,4-dinitrophenyl-hydrazine (Wako Pure Chemical, special grade) were used to prepare the DNPH derivatization reagent. Water was purified with a PURELAB flex3 (Veolia Water Solution & Technologies Japan, Tokyo, Japan) and had a specific resistance of 18.2 MΩ.

The optimized mobile phase contained 70% (v/v) methanol and was passed through the separation column at a flow rate of 1.0 mL/min. The measurement temperature and sample injection volume were 30 °C and 100 µL, respectively. The signal wavelength of the diode array detector was set to 365 nm.

When comparing with the official analysis method, the mobile phase contained 50% (v/v) acetonitrile and was passed through the separation column at a flow rate of 1.0 mL/min. The measurement temperature and sample injection volume were the same as the proposed method. The signal wavelength of the diode array detector was set to 360 nm.

The reaction scheme for the methyl acetoacetate derivatization reaction is shown in Fig. 1. The derivatization reagent was prepared as follows: 2.0 mL of methyl acetoacetate and 7.0 g of ammonium acetate were added to less than 100 mL of 70% (v/v) methanol (mobile phase) into a beaker. After complete dissolution, resultant solution was transferred to a 100 mL volumetric flask and more mobile phase was added until the volume level reached the marked line. Then, 3.0 mL of the aforementioned derivatization reagent and 3.0 mL of the water sample to be analyzed were mixed in a stoppered test tube. After stirring, the mixture was heated at 60 °C in a water bath for 20 min. The obtained sample solution was cooled with ice for approximately 3 min before 100 µL of the sample solution were injected into the HPLC.

On the other hand, the derivatization reagent was prepared as follows: 0.1 g of 2,4-dinitrophenyl-hydrazine was added to less than 100 mL of 50% (v/v) acetonitrile (mobile phase) in a beaker. After complete dissolution, the resultant solution was transferred to a 100 mL of volumetric flask and more mobile phase was added until the volume level reached the marked line. Then, 0.5 mL of the aforementioned derivatization reagent and 10.0 mL of the water sample to be analyzed were mixed in a stoppered test tube. After

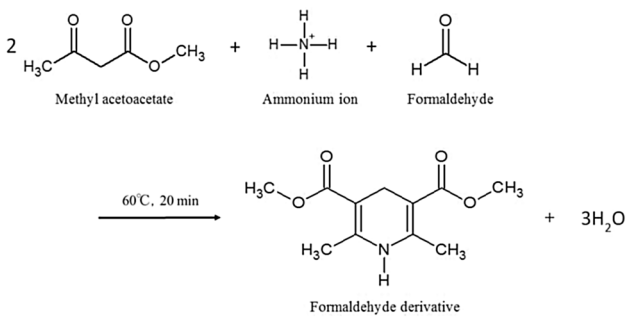

Fig. 1 Derivatization reaction of methyl acetoacetate

adding 0.2 mL of 20% (v/v) phosphoric acid, the mixture was allowed to stand for 20 min at room temperature, and then 100 µL of the resultant sample solution were injected into the HPLC.

Water samples used in this study included tap water, river water, and wastewater generated after a chemical experiment. As a pretreatment for each water sample, they were first filtered through a No.5A filter paper (0.1 mm pore size, Advantec Toyo, Tokyo, Japan), and suction filtration was then carried out using a 0.45 µm pore membrane filter manufactured by the same company. In the HCHO recovery test, 0.04–0.20 mg/L solutions of HCHO were prepared by diluting with each water sample, and the derivatization procedure was performed. Derivatization was carried out as soon as possible after sampling to avoid decomposition of HCHO in each water sample.

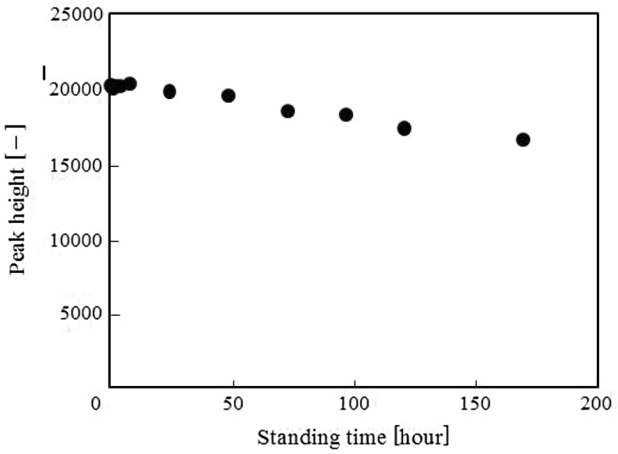

Fig. 2 Stability of the HCHO derivative

Results and Discussion

According to our previous studies using the acetylacetone derivatization method, the analytical precision of the HCHO detection was high enough for application (Ishikawa et al. 2014). When applying this derivatization method to analyze HCHO in tap water, satisfactory results were obtained. However, the recovery rate of HCHO was unstable (44%–136%) when this method was used for analyzing river water samples. The calibration curve and the recovery rate of HCHO were not satisfactory when ethyl acetoacetate, which has a structure that closely resembles acetylacetone, was used. Furthermore, the reproducibility of the peak intensity at each concentration of HCHO was also poor. It is considered that one of the main reasons for showing a poor result is the influence of coexisting components in river water. Based on these results, methyl acetoacetate was chosen as the HCHO derivatization reagent for this study.

When using methyl acetoacetate as the derivatization reagent, the peak height of the HCHO derivative changed with time. Therefore, we used these results to confirm the stability of the derivative. As shown in Fig. 2, the peak height of the HCHO derivative did not change significantly until 48 h, but gradually decreased thereafter. The peak height of the HCHO derivative suddenly decreased after 168 h. Therefore, it is best to use the derivatization reagent within 48 h after preparation.

The chromatograms of HCHO derivative following the proposed method and the official analysis method (DNPH derivatization) are shown in Fig. 3. The HCHO derivative peak was detected within 4 min for the proposed method. Although a peak derived from excess derivatizing reagent was detected at approximately 2 min, it did not affect the detection of HCHO derivative. In contrast, the HCHO

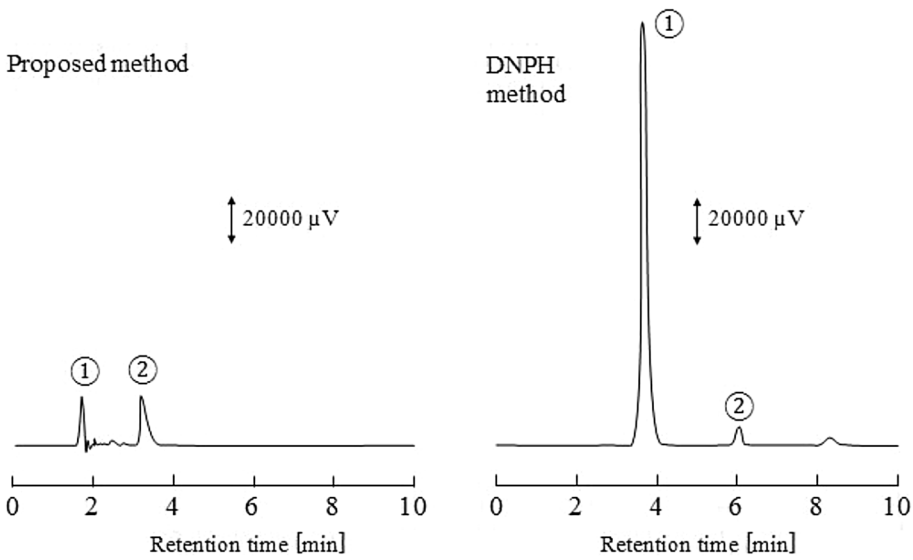

Fig. 3 Chromatograms of the HCHO derivative. ① Excessive derivatization reagent ② HCHO derivative

derivative peak was detected within 6 min for the DNPH derivatization method. Although the peak derived from an excessive DNPH reagent was detected at approximately 3 min, it did not affect the detection of HCHO derivative either. The proposed method has a high detection sensitivity for HCHO derivative and a shorter retention time than the DNPH method. Considering the aforementioned results, the proposed method is competitive with the DNPH method.

Analytical precision of the HCHO detection was confirmed both in the proposed method and the official analysis method. Table 1 shows the linearities of the calibration curves, together with the reproducibility and detection/quantitation limits for each derivatization method. The calibration curves obtained from the peak heights were plotted using three replicates for each level of concentration (0–0.16 mg/L). Both calibration curves were linear with correlation coefficients of 0.9998. The relative standard deviations (R.S.D.) of the peak heights from ten replicates at each concentration were below 0.7%. The proposed method showed a lower R.S.D. value than the DNPH method despite of the lower HCHO concentrations used. The detection and quantitation limits calculated from the slope of the calibration curves were around 1.0 and 3.0 µg/L, respectively. There was no noticeable difference in the detection and quantitation limit of HCHO obtained by both methods. Because different mobile phases are used, the detection sensitivity of HCHO seems to be different comparing the chromatograms shown in Fig. 3. From the viewpoint of these results, the proposed method is comparable to the official method.

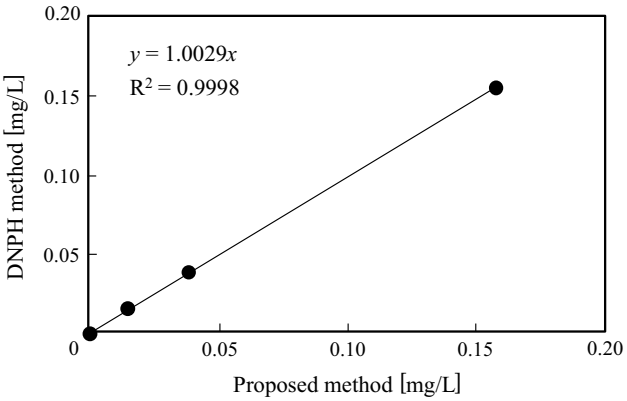

Fig. 4 Comparison of each method. DNPH, 2,4-dinitrophenylhydrazine. Samples are analyzed in triplicated. X-axis and Y-axis are the quantitative value of formaldehyde in proposed and DNPH method, respectively

A recovery test was performed for the water samples to confirm the reliability of proposed method, and to compare this with the official analysis method. Several HCHO standard solutions with known concentrations (0.04–0.20 mg/L) diluted in each water sample were prepared. The recovery

Table 1 Analytical precision of the HCHO derivative

	Range (mg/L)	R^2	Conc. (µg/L)	R.S.D. (%)	LOD (µg/L)	LOQ (µg/L)
Proposed method	0–0.16	0.9998	8.0	0.29	0.96	3.16
DNPH method	0–0.16	0.9998	40.0	0.64	0.91	3.00

R.S.D., relative standard deviation; LOD, limits of detection; LOQ, limits of quantification; DNPH, 2,4-dinitrophenylhydrazine

Table 2 Comparison of HCHO recovery test

Recovery rate (%)	Added HCHO (mg/L)				
	0.04	0.08	0.12	0.16	0.20
Proposed method					
Tap water A	100 ± 2.3	101 ± 1.9	102 ± 1.6	105 ± 2.1	104 ± 1.5
Tap water B	108 ± 11.0	108 ± 5.0	95 ± 2.6	100 ± 2.6	103 ± 1.1
River water A	107 ± 4.7	104 ± 1.3	107 ± 1.1	102 ± 0.3	99 ± 1.4
River water B	94 ± 1.5	104 ± 1.1	104 ± 1.5	106 ± 0.5	108 ± 0.4
Waste water	106 ± 14.0	98 ± 5.9	97 ± 5.1	98 ± 3.2	103 ± 3.6
DNPH method					
Tap water A	93 ± 5.9	98 ± 1.4	100 ± 0.2	98 ± 2.8	98 ± 1.3
Tap water B	97 ± 7.9	120 ± 1.0	103 ± 0.4	100 ± 2.7	96 ± 0.9
River water A	95 ± 1.5	113 ± 1.1	98 ± 0.3	106 ± 0.8	104 ± 0.4
River water B	88 ± 5.0	91 ± 0.9	93 ± 0.2	92 ± 2.8	94 ± 1.2
Waste water	95 ± 5.1	100 ± 1.2	103 ± 0.3	102 ± 2.2	104 ± 1.2

Samples are analyzed in triplicated (Mean ± S.D.)

DNPH, 2,4-dinitrophenylhydrazine; HCHO, formaldehyde

Table 3 Analytical results of HCHO in several water samples

HCHO conc. (μg/L)	Tap water A	Tap water B	River water A	River water B	Waste water
Proposed method	7.9 ± 0.1	< LOQ	< LOQ	< LOQ	17.0 ± 0.2
DNPH method	7.8 ± 0.1	< LOQ	< LOQ	< LOQ	16.7 ± 0.1

Samples are analyzed in triplicated (Mean ± S.D.)

LOQ, limits of qualification; DNPH, 2,4-dinitrophenylhydrazine; HCHO, formaldehyde

rates of HCHO obtained from the peak heights are shown in Table 2. The HCHO recovery rate for the proposed method and the DNPH method were 93%–108% and 88%–120%, respectively. It was proved that the variation of HCHO recovery rate in proposed method was small. Although many cations and anions are present as coexisting components in the water samples, the Hantzsch reaction was apparently unaffected by them.

We checked the correlation between the proposed method and the DNPH method to confirm the reliability. A calibration curve was prepared using 0–0.16 mg/L HCHO standard solutions diluted with the tap water A, and the correlation of both methods was confirmed. The horizontal axis and the vertical axis shown in Fig. 4 are the HCHO concentrations calculated from the proposed method and the DNPH method, respectively. The observed correlation was very high ($R_2 = 0.9998$); the HCHO concentrations obtained from both methods were closed to the nearly theoretical values ($y = 1.0029x$), and the slope of the straight line was 1.0029.

The proposed method was applied to the analysis of HCHO levels in real water samples. In addition, we measured HCHO levels in the same water samples using the DNPH method for comparison. Table 3 shows the analytical results for HCHO levels in the real water samples using both methods. HCHO below the tap water quality standard (0.08 mg/L) was detected from tap water A, and both methods showed nearly the same quantitative values. Although the HCHO peak was detected for tap water B, river water A, and river water B, the quantitative values were below the quantitation limit. A higher HCHO concentration was detected in experimental waste water. Although a waste water treatment facility is provided in our office building, mainly inorganic substances such as metal ions are treated; organic substances are not treated perfectly. Therefore, a higher concentration of HCHO was detected for the waste water than for the other water samples.

High-performance liquid chromatography using UV detection with methyl acetoacetate derivatization was developed for the determination of HCHO levels in several water samples. When methyl acetoacetate was used as the derivatization reagent, both the linearity of the calibration curve and the reproducibility of the HCHO peak intensity were excellent. The derivatization reagent was stable for up to 48 h under refrigerated storage. In addition, no change in detection sensitivity was observed during the same period. The proposed method has a high detection sensitivity for HCHO and the retention time is shorter than that of the DNPH method. There was no significant difference in the quantitative values of HCHO between the proposed method and the DNPH method. The correlation of both methods was also good. Based on the results presented here, we can say that the proposed method is competitive with the DNPH method.

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Compliance with Ethical Standards

Conflict of Interests The authors declare that they have no conflict of interest.

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