J Occup Health 2009; 51: 386–390

Short Communication

Optimization of the Determination of Ortho-phthalaldehyde in Air by Derivatization with 2,4-dinitorophenylhyrazine (DNPH)

Shinobu YAMAMOTO[1], Akito TAKEUCHI[2], Yoshihiro SUZUKI[3], Yoko ENDO[3], Yoko EITAKI[2], Hiroshi KATAGIRI[4], Jun TAKAHASHI[1], Tomojiro YASUGI[1], Tomoko TAKIGAWA[5] and Keiki OGINO[5]

[1]Chugoku and Shikoku Regional Safety and Health Service Center, Japan Industrial Safety and Health Association, [2]Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, [3]Clinical Research Center for Occupational Poisoning, Tokyo Rosai Hospital, Japan Labour, Health, and Welfare Organization, [4]Department of Health Science, Kitasato University, School of Allied Health Sciences and [5]Department of Public Health, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

Key words: Derivatization, 2,4-dinitorophenylhydrazine, Ortho-phthalaldehyde

Endoscopic equipment and other medical devices are currently sterilized with three types of sterilizing agent, glutaraldehyde (GA), ortho-phthalaldehyde (OPA) and peracetic acid. OPA is considered a powerful, high-level disinfectant because it is more effective against GA-resistant mycobacteria, is less irritating and has a shorter disinfection time than GA, and does not require any activation step[1].

Due to the development of allergic dermatitis and asthma among medical workers exposed to GA[2, 3], the Japanese Ministry of Health, Labour, and Welfare[4] recommended in February 2005 that GA exposure in sterilization units should be kept below 0.05 ppm. OPA as a safer alternative to GA has therefore been more frequently utilized for sterilization in an increasing number of Japanese hospitals. However, allergic disorders have also been reported among medical workers using OPA[5].

OPA concentrations in work environments have been reported to be lower than those of GA[6–8], indicating a need for reliable methods for measuring OPA. Moreover, the safety of medical workers requires strict control of OPA, maintaining low concentrations in air.

Received Mar 2, 2009; Accepted May 14, 2009
Published online in J-STAGE Jun 18, 2009
Correspondence to: A. Takeuchi, Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, 2–3–8 Tosabori, Nishi-ku, Osaka 550-0001, Japan (e-mail: a-takeuchi@jisha.or.jp)

Measurements of OPA in the air of work environments have been quantitatively determined by HPLC after pre-column derivatization with 2,4-dinitorophenylhydrazine (DNPH)[5, 8–10]. We recently[11, 12] found that the hydrazone derivatives of OPA with DNPH are composed of several different chemical forms, the major one being bis-DNPhydrazone. We also found that the rate of formation and the relative abundance of the hydrazone derivatives of OPA with DNPH depend on the eluting conditions, such as phosphoric acid concentration or reaction time, during the extraction of OPA from DNPH-silica cartridges. In this report we describe the characteristic profiles of the hydrazone derivatives of OPA with DNPH, as well as the optimal analytical conditions to measure OPA in the air of work environments.

Materials and Methods

Materials

Ortho-phthalaldehyde (OPA), 2,4-dinitrophenylhydrazine (50% aqueous solution, DNPH) and phosphoric acid of guaranteed reagent grade were purchased from Tokyo Chemical Industry (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Sigma-Aldrich Japan (Tokyo, Japan), respectively. HPLC grade acetonitrile was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Water was purified with a Milli-Q water system (Millipore, Bedford, MA, USA). DISOPA solution (0.55% OPA) was purchased from Johnson and Johnson (Tokyo, Japan).

A series of phosphoric acid/acetonitrile solutions was prepared by dissolving calculated amounts of phosphoric acid in acetonitrile. A standard solution of OPA was prepared by dissolving a calculated amount of OPA in acetonitrile (2 μg/m*l*). DNPH solution was prepared by dissolving a calculated amount of DNPH, which recrystallized with acetonitrile, in acetonitrile (0.2 mg/m*l*).

DNPH-silica cartridges of LpDNPH S10 and GL-Pak mini AERO DNPH for OPA sampling were purchased from Supelco Inc. (Bellefonte, PA, USA) and GL Sciences Inc. (Tokyo, Japan), respectively. Sampling was performed by introducing air into the DNPH-silica cartridge with a SKC Air Check 2000 (SKC Inc., Eighty Four, PA, USA).

Characterization of the hydrazone derivatives of OPA with DNPH by HPLC-MS/MS

Ten microliters of a reaction mixture of OPA with DNPH was injected into an HPLC system (HP1100, Agilent, USA) connected to an Inertsil C8-3 column (250 × 4.6 mm i.d., GL Science, Tokyo, Japan) under the following conditions: mobile phase of 70% acetonitrile and 30% water; flow rate, 1.0 m*l*/min; column temperature, 26°C. The hydrazone derivatives of OPA with DNPH were detected using an ion trap mass analyzer

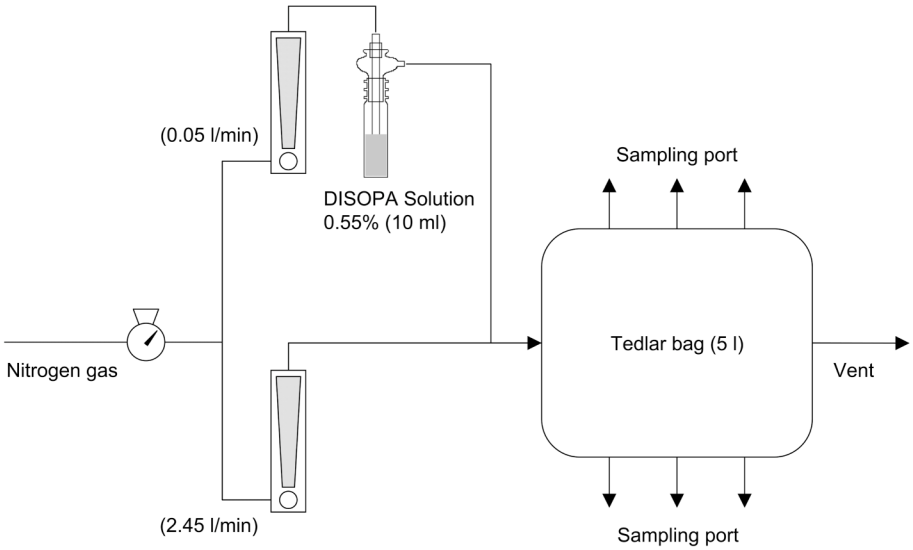

Fig. 1. Schematic illustration of the test gas generator.

type of LC-MS/MS (LCQ, Thermoquest, Japan). The mass-spectra of the derivatives was obtained by atmospheric pressure chemical ionization (APCI) in negative-ion mode. Collision induced dissociation (CID) was used for MS^n spectrometry.

Derivatization of OPA with 2,4-dinitrophenylhydrazine in phosphoric acid solutions

The hydrazone derivative of OPA with DNPH were analyzed with a TOSHO 8020 Series HPLC system (TOSHO, Tokyo, Japan), and were monitored with a UV spectrophotometric detector at 383 nm (SPD-6A, Shimadzu, Kyoto, Japan). The analytical column and the mobile phase were the same as those used for the LC-MS/MS analysis. The column temperature was kept at 40°C and a 20-μl aliquot of the sample solution was injected.

To determine the optimal reaction conditions for derivatization of OPA with DNPH, we assessed the effect of phosphoric acid concentration and the transformation of OPA mono-DNPhydrazone to OPA bis-DNPhydrazone using a standard solution of OPA and a UV detector.

1. Effect of phosphoric acid concentration

To examine the effect of phosphoric acid concentration on the derivatization reaction, a 50-μl aliquot of OPA standard solution (2 μg/m*l*) was added to each test tube containing 0.9 m*l* of DNPH solution (0.2 mg/m*l*) and 50 μl of phosphoric acid/acetonitrile solution, containing different concentrations of phosphoric acid, ranging from 0.005% to 20% (v/v). Each tube was vortexed for 30 s and allowed to stand for 30 min at room temperature. The final concentration of phosphoric acid in the test tubes ranged from 0.00025 to 1.0% (v/v). The amounts of hydrazone derivatives of OPA with DNPH in each test tube were determined by HPLC analysis.

2. Transformation of OPA mono-DNPhydrazone to OPA bis-DNPhydrazone

Fifty microliters of OPA standard solution (2 μg/m*l*) was added to a test tube containing 0.9 m*l* of DNPH solution (0.2 mg/m*l*) and 50 μl of phosphoric acid (0.1%, v/v)/acetonitrile solution. The tube was vortexed for 30 s and allowed to stand at room temperature. At various time points, ranging from 0 to 10.5 h, aliquots were withdrawn, and the amounts of OPA mono- and OPA bis-DNPhydrazone were determined by HPLC analyses.

Generation of OPA test gas and collection with DNPH-silica cartridge

OPA test gas was generated with a gas generator, as shown in Fig. 1. Nitrogen gas, at a flow rate of 0.05 *l*/min, was introduced to the impinger containing 10 m*l* of DISOPA solution, and the dynamically generated OPA gas was diluted with nitrogen gas, at a flow rate of 2.45 *l*/min, into a Tedlar® bag (5 *l*), which was used as an exposure chamber, at ambient temperature. Due to the relatively low vapor pressure of OPA, 30 min was necessary to stabilize the OPA gas concentration in the Tedlar bag. The relative standard deviation of OPA gas concentration was 16% for intra-day reproducibility.

The OPA test gas was passed through the DNPH-silica cartridge for 10 min at a flow rate of 1.0 *l*/min. The OPA-DNPH derivatives collected in the DNPH-silica cartridge were extracted immediately with 5 m*l* of acetonitrile. A 25-μl aliquot of phosphoric acid/

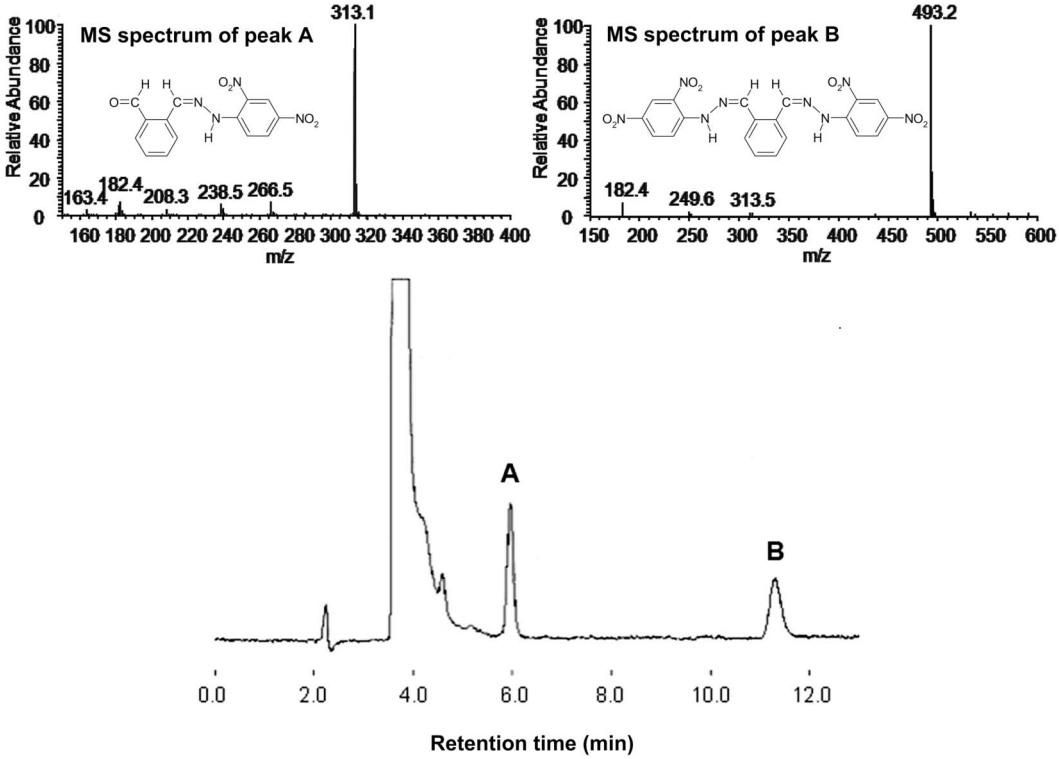

Fig. 2. HPLC-UV chromatogram and ion trap APCI mass spectra of the test solution, in which OPA standard solution was reacted with DNPH. Peak (A), OPA mono-DNPhydrazone; Peak (B), OPA bis-DNPhydrazone.

acetonitrile solution (2%, v/v) was added to 475 μl of the eluant solution. The sample was allowed to stand at room temperature for 0 to 4 h and aliquots were subsequently analyzed by HPLC-UV.

In this HPLC analysis, the peak corresponding to OPA mono-DNPhydrazone overlapped with the tail of the large peak due to the derivatizing reagents. Therefore, the time-course of the derivatization reaction was traced by measuring OPA bis-DNPhydrazone.

Results and Discussion

Characterization of hydrazone derivatives of OPA with DNPH by LC-MS/MS

The HPLC-UV chromatogram and the ion trap APCI mass spectra of the test solution, in which OPA standard solution was reacted with DNPH, are shown in Fig. 2. Analysis of the ion trap APCI mass spectra showed that m/z=313.1 and m/z=493.2 were the precursor ions of peaks (A) and (B), respectively. Both peaks have mass fragments of m/z=182.4, which originated from DNPH. Peaks (A) and (B) correspond to OPA mono-DNPhydrazone and OPA bis-DNPhydrazone, respectively. These findings are in agreement with those of a previous HPLC analysis of the eluate from a DNPH-silica cartridge after passage of OPA standard vapor[13], which found one peak each for OPA mono- and OPA bis-DNPhydarazone.

Derivatization of OPA with 2,4-dinitrophenylhydrazine in phosphoric acid solutions

In the presence of catalytic amounts of acid, carbonyl compounds, including carboxylic acids, aldehydes and ketones, react with DNPH to form hydrazones[13]. Although non-volatile phosphoric acid is the usual catalytic agent for these reactions, the optimal acid concentration for the reaction of a carbonyl compound with DNPH differs for each compound, and the optimal concentration for derivatization of OPA has not yet been reported. We therefore assessed the optimum concentration of phosphoric acid and reaction time to reach a plateau value for the ratio of OPA mono-DNPhydrazone to OPA bis-DNPhydrazone. The effect of phosphoric acid concentration on the derivatization reaction of OPA with DNPH is shown in Fig. 3. The peak area of mono-derivative increased with increasing phosphoric acid concentration, reaching a maximum value at 0.0075%, but decreasing thereafter. In contrast, the peak area of the bis-derivative began to increase dramatically at about 0.0075% phosphoric acid and plateaued at a concentration higher than 0.075%. These results suggest that the amounts of mono- and bis-derivatives produced by the reaction of OPA with DNPH

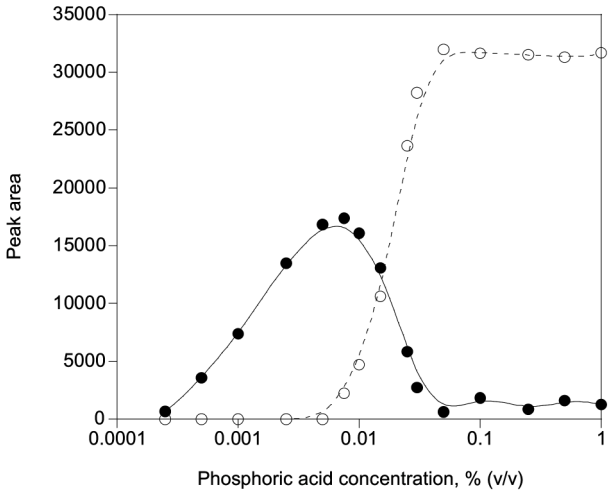

Fig. 3. Effect of phosphoric acid concentration on the formation of hydrazone derivatives. A 50-μl aliquot of phosphoric acid/acetonitrile solution, the phosphoric acid concentration of which ranged from 0.005 to 20% (v/v), was added to a test tube containing 50 μl of OPA standard solution and 0.9 m*l* of DNPH solution. The final concentration of phosphoric acid in test tubes ranged from 0.00025 to 1.0% (v/v). OPA mono-DNPhydorazone (●), OPA bis-DNPhydrazone (○).

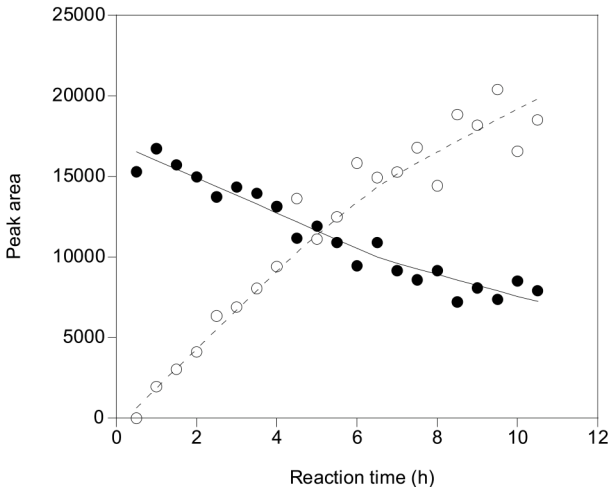

Fig. 4. Transformation of OPA mono-DNPhydrazone derivatives to OPA bis-DNPhydrazone derivatives in acetonitrile solution. The reaction time was altered from 0 to 10.5 h. OPA mono-DNPhydrazone (●), OPA bis-DNPhydrazone (○).

depend on the concentration of phosphoric acid.

Figure 4 shows the time dependence of the transformation of OPA mono-DNPhydrazone to OPA bis-DNPhydrazone. Although no appreciable amount of the bis-derivative was detected immediately after the addition of 0.1% phosphoric acid, the amount of the bis-derivative increased over time, while the amount of the mono-derivative decreased.

These results suggest that the reaction of 1 mol of OPA with 1 mol of DNPH first yields the mono-derivative, later forming the bis-derivative by reacting with another 1 mol of DNPH and agree with findings using OPA standard vapor[10].

To minimize analytic errors, it is important to reach equilibrium in OPA bis-DNPhydrazone formation, and the optimum concentration of phosphoric acid we determined, is expected to reduce the time needed to reach equilibrium.

Generation of OPA test gas and collection with DNPH-silica cartridges

Based on the measurements of the phosphoric acid contents of various commercially available DNPH-silica cartridges, the optimal acid concentration and reaction time have been determined for the analysis of ketone-2,4-dinitrophenylhydrazones by HPLC[13]. The optimal concentration of phosphoric acid and the reaction time necessary for the determination of OPA are expected to be different from those for ketones. It is also unclear

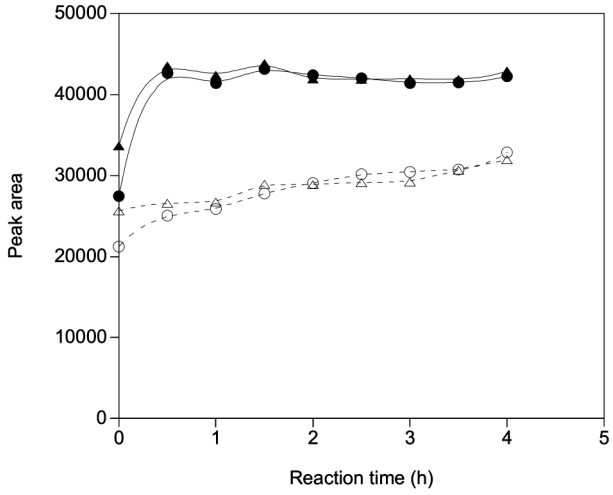

Fig. 5. Variations of peak areas of OPA bis-DNPhydrazone in eluates from DNPH-silica cartridges in the presence (●: LpDNPH S10, ■: GL-Pak mini AERO DNPH) or absence (○: LpDNPH S10, □: GL-Pak mini AERO DNPH) of additional phosphoric acid. The DNPH-silica cartridges used in this study were LpDNPH S10 and GL-Pak mini AERO DNPH, respectively.

whether the formation of OPA bis-DNPhydrazone is complete in the presence of phosphoric acid in DNPH-silica cartridges.

We therefore assessed the formation of OPA bis-DNPhydrazone using two kinds of commercially available DNPH-silica cartridges, after sampling of OPA

test gas. Figure 5 shows the HPLC analyses of OPA bis-DNPhydrazone in the eluates of DNPH-silica cartridge. The formation of OPA bis-DPNhydrazone varied according to the presence or absence of phosphoric acid in the eluates and with the time of reaction. When phosphoric acid was added to the eluate, the peak areas of OPA bis-DNPhydrazone reached a plateau after 30 min for both DNPH-silica cartridges. In the absence of added phosphoric acid, however, the peak areas gradually increased over time, up to at least 4 h after elution. Relative to the peak area of OPA bis-DNPhydrazone in the presence of phosphoric acid at 4 h, the peak area in the absence of phosphoric acid at 4 h was approximately 80%. These results indicate that the derivatization reaction is not completed in the cartridge and continues in the effluent. The presence of phosphoric acid markedly accelerates the reaction rate of OPA mono-DNPhydrazone to OPA bis-DNPhydrazone. While a previous study[10] reported that the transformation reaction of the mono- to the bis-derivative occurred rapidly in acetonitrile and was completed in 4 h, we found that this transformation reaction was not completed within 4 h. This discrepancy may be due to differences in the phosphoric acid concentration in the cartridges. We used commercially available DNPH-silica cartridges, whereas the previous study used hand-made cartridges, with different concentrations of phosphoric acid.

Conclusion

We have determined the optimal analytical conditions for the measurement of OPA in air of work environments using commercially available DNPH-silica cartridges. OPA mono- and OPA bis-DNPhydrazone of OPA-DNPH were found in the effluent from these DNPH-silica cartridges, to which the test atmosphere had been introduced. The HPLC analysis of OPA shows that measurements of OPA bis-DNPhydrazone are suitable for obtaining reliable data for OPA in air. We also demonstrated that the degree of transformation of OPA mono-DNPhydrazone to OPA bis-DNPhydrazone depends on the reaction time and the concentration of phosphoric acid in the sample solution. To obtain complete formation of OPA bis-DNPhydrazone, (1) a 25-μl aliquot of phosphoric acid (2%, v/v) should be added to 475 μl of effluent from DNPH-silica cartridges and (2) the solution should be allowed to stand for more than 30 min at room temperature in order to reach equilibrium in the reaction between OPA and DNPH.

The present method for the determination of OPA is based on practically complete formation of OPA bis-DNPhydrazone and is expected to minimize analytical errors.

Acknowledgments: The authors thank Dr. Terumasa Nakamura for feedback on the manuscript.

References

1) Hession SM. Endoscope disinfection by ortho-phthalaldehyde in a clinical setting: An evaluation of reprocessing time and costs compared with glutaraldehyde. Gastroenterol Nurs 2003; 26: 110–4.
2) American Conference of Governmental Industrial Hygienists. Glutaraldehyde Documentation of the TLVs and BEIs with other world wide occupational exposure values. Cincinnati (OH): ACGIH; 2005 (CD-ROM).
3) Takigawa T, Endo Y. Effects of glutaraldehyde exposure on human health. J Occup Health 2006; 48: 75–87.
4) Labour Standards Bureau of Japanese Ministry of Health Law. Preventive measures against health hazard of workers caused by GA exposure in medical facilities. Tokyo: Japanese Ministry of Health, Labour and Welfare; 2005.
5) Fujita H, Ogawa M, Endo Y. A case of occupational bronchial asthma and contact dermatitis caused by ortho-phthalaldehyde exposure in a medical worker. J Occup Health 2006; 48: 413–6.
6) Marena C, Lodola L, Marone Bianco A, et al. Monitoring air dispersed concentrations of aldehydes during the use of ortho-phthalaldehyde and glutaraldehyde for high disinfection of endoscopes. G Ital Med Lav Ergon 2003; 25: 131–6.
7) Fujita H, Sawada Y, Ogawa M, Endo Y. Health hazards from exposure to ortho-phthalaldehyde, a disinfectant for endoscopes, and preventive measures for health care workers. Sangyo Eiseigaku Zasshi 2007; 49: 1–8 (in Japanese).
8) Miyajima K, Tabuchi T, Kumagai S. Occupational health of endoscope sterilization workers in medical institutions in Osaka Prefecture. Sangyo Eiseigaku Zasshi 2006; 48: 169–75 (in Japanese).
9) Yamamoto T, Katagiri H, Suzuki T, Aizawa K, Kadowaki T. About examination of the measuring method of Ortho-phthalaldehyde, and measurement of the medical spot. Sangyo Eiseigaku Zasshi 2003; 45: 614 (in Japanese).
10) Uchiyama S, Matsushima E, Tokunaga H, Otsubo Y, Ando M. Determination of orthophthalaldehyde in air using 2,4-dinitrophenylhydrazine-impregnated silica cartridge and high-performance liquid chromatography. J Chromatography A 2006; 1116: 165–71.
11) Kubota R, Inoue Y, Nishinakagawa S, Morita Y, Yukita K, Endo Y. Underlying examination about the passive sampling method of o-phthalaldehyde. Jpn J Occup Med Traumatol 2005; 53: 221 (in Japanese).
12) Takeuchi A, Yamamoto S, Endo Y, et al. Underlying examination about measurement of o-phthalaldehyde in a work-environment—Effects of the acid level in the DNPH derivatization—. Sangyo Eiseigaku Zasshi 2007; 49: 145 (in Japanese).
13) Uchiyama S, Kaneko T, Tokunaga H, Ando M, Otsubo Y. Acid-catalyzed isomerization and decomposition of ketone-2,4-dinitrophenylhydrazones. Anal Chim Acta 2007; 605: 198–204.