Development of a measurement method to determine the ceiling exposure concentration of \textit{ortho}-phthalaldehyde handling workers

Shinobu Yamamoto$^{1,2,}$ | Akito Takeuchi$^{3}$ | Toru Ishidao$^{1}$ | Hiroaki Ohkuma$^{4}$ | Masayoshi Ichiba$^{2}$ | Hajime Hori$^{1}$

$^{1}$Department of Environmental Measurement and Control, University of Occupational and Environmental Health, Kitakyushu, Japan
$^{2}$Department of Social Medicine, Saga University, Saga, Japan
$^{3}$Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, Osaka, Japan
$^{4}$Komyo Rikagaku Kogyo K.K., Kawasaki, Japan

Correspondence
Shinobu Yamamoto, Department of Environmental Measurement and Control, University of Occupational and Environmental Health, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan.
Email: shinobu-y@health.uoeh-u.ac.jp

Abstract

Objectives: The purpose of this research was to develop and validate an analytical method for rapid determination of the exposure of workers to \textit{ortho}-phthalaldehyde (OPA) at the ceiling threshold concentration.

Methods: A 2,4-dinitrophenylhydrazine (DNPH)-silica cartridge was chosen as a sampler. OPA collected by the DNPH-silica cartridge was subsequently extracted with 5 mL of acetonitrile. A 50-µL aliquot of phosphoric acid/acetonitrile solution (2%, v/v) was added to 950 µL of the extraction solution and allowed to stand for 30 minutes at room temperature. This solution was then analyzed by high-performance liquid chromatography tandem mass spectrometry. The basic characteristics of the proposed method, such as recovery, repeatability, limit of quantification, and storage stability of the samples, were examined.

Results: The overall recoveries of OPA from OPA-spiked DNPH-silica cartridges were 93.6%-100.1% with relative standard deviations, representing the repeatability, of 1.5%-10.8%. The limit of quantification was 0.165 ng/sample. The recovery of OPA from DNPH-silica cartridges after 5 days of storage in a refrigerator exceeded 95%.

Conclusions: The proposed method enabled the determination of the OPA concentration corresponding to the Threshold Limit Value-Ceiling of 0.1 ppb recommended by the American Conference of Governmental Industrial Hygienists, with a minimum sampling time of 18 seconds (corresponding to a sampling volume of 300 mL at 25°C and 1 atm). Thus, this method will be useful for estimating worker exposures to OPA.

KEYWORDS

air sampling method, HPLC-MSMS, \textit{ortho}-phthalaldehyde, Threshold Limit Value-Ceiling, workplace air

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1 | INTRODUCTION

ortho-phthalaldehyde (OPA), which has a structure consisting of two aldehyde groups attached to adjacent carbon atoms in a benzene ring, is widely used as a sterilant and disinfectant for medical equipment such as endoscopes. OPA can cause eye and respiratory irritation, dermatitis, and respiratory sensitization in exposed medical workers and patients. Although an occupational exposure limit (OEL) for OPA has not been recommended by the Japan Society for Occupational Health (JSOH), in 2019, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended a Threshold Limit Value-Ceiling (TLV-C) of 0.1 ppb. Therefore, because the target concentration is so low and the sampling should be performed in as short time as possible, an analytical method with very high sensitivity is desired for OPA determination.

Several methods have been reported for the determination of OPA concentrations in workplace air. In these methods, OPA in workplace air was sampled using a 2,4-dinitrophenylhydrazine (DNPH)-silica cartridge and then analyzed using high-performance liquid chromatography (HPLC). However, the sensitivity of these methods is not sufficient for the determination of the ceiling exposure concentration. Our previous study showed the hydrazone derivatives formed by reaction of OPA with DNPH consisted of mono-DNPhydrazone and bis-DNPhydrazone. In addition, we found that the rate of formation and the relative abundance of the hydrazone derivatives of OPA depended on the elution conditions, such as phosphoric acid concentration or reaction time, during the extraction of OPA from the DNPH-silica cartridge. Moreover, we determined the optimal sample preparation procedure for obtaining reliable data via HPLC analysis.

The aim of this study was to develop and validate an analytical method for determining the exposure of OPA-handling workers at the ceiling exposure concentration. To achieve this, we prepared samples according to our reported procedure and proposed a detection method with high sensitivity based on high-performance liquid chromatography-tandem mass spectrometry (HPLC-MSMS).

2 | MATERIALS AND METHODS

2.1 | Materials

OPA (special use grade) was purchased from Tokyo Chemical Industry. A hydrazone derivative of OPA (OPA bis-DNPH, 10 µg/mL acetonitrile solution) was purchased from Merck. Phosphoric acid (HPLC grade) was purchased from Nacalai Tesque, Inc. Acetonitrile and formic acid (HPLC/MS grade) were purchased from Fujifilm Wako Pure Chemical Corporation. Water was purified using a Milli-Q water system from Merck.

DNPH-silica cartridges (400 mg containing 1 mg of DNPH, 815H) for OPA sampling were purchased from Komyo Rikagaku Kogyo KK. Sampling was performed by introducing air into the DNPH-silica cartridge with a GSP-400FT sampling pump (Gastec Corporation). Standard solutions of OPA and OPA bis-DNPH were prepared in acetonitrile.

2.2 | HPLC instrumentation and conditions

HPLC separation was conducted using a Shimadzu Nexera UHPLC/HPLC system consisting of a DGU-20AR degasser, two LC-30AD pumps, an SIL-30AC autosampler, and a CTO-30A column oven (Shimadzu Scientific Instruments). An InertSustain C18 column (2.1 mm × 150 mm; 2 µm; GL Sciences Inc) was used and the column temperature was 40°C. The mobile phase consisted of a 70/30 (v/v) mixture of acetonitrile and 0.1 vol% formic acid in water. The flow rate was 0.2 mL/min and the injection volume was 2 µL.

2.3 | MSMS instrumentation and conditions

Qualitative and quantitative determination of the target compounds was conducted using an LCMS-8030 triple quadrupole mass spectrometer with an electrospray ionization (ESI) source (Shimadzu) in negative ion mode. The analyses parameters were as follows: interface voltage = 4.5 kV; interface temperature = 350°C; resolution line temperature = 250°C; nebulizer gas flow = 3.00 L/min; block heater temperature = 400°C; and drying gas flow = 15.00 L/min. Collision-induced dissociation (CID) was performed with argon gas. Multiple reaction monitoring (MRM), optimized using OPA bis-DNPH standard solutions, was applied for analysis of the OPA bis-DNPH-derived fragment combination.

2.4 | Sample preparation

After sampling, OPA mono-DNPH and OPA bis-DNPH on the DNPH-silica cartridge were extracted with 5 mL of acetonitrile. A 50 µL aliquot of phosphoric acid/acetonitrile solution (2%, v/v) was added to 950 µL of the extraction solution. The sample was allowed to stand at room temperature for 30 minutes, and aliquots were subsequently analyzed by HPLC-MSMS.

2.5 | Method validation

The OPA standard solution was diluted with acetonitrile and then 100 µL of the diluted solution was spiked onto the
DNPH-silica cartridge. Simultaneously, clean air was introduced into the DNPH-silica cartridge at a flow rate of 1 L/min for 10 minutes. In a preliminary experiment to confirm breakthrough, two DNPH-silica cartridges connected in series were spiked with the OPA standard solution and then clean air was introduced.

For the recovery test (n = 5), the spiked amounts ranged from 0.509 to 509 ng. Recovery was determined by comparing the OPA bis-DNPH peak area in the spiked DNPH-silica cartridges with those of OPA bis-DNPH standard solutions.

For the storage stability tests (n = 5), two different amounts of OPA (12.4 and 517.6 ng) were spiked onto DNPH-silica cartridges. After air was drawn through the spiked DNPH-silica cartridge at a flow rate of 1 L/min for 10 minutes, they were sealed and stored in a refrigerator (4°C) for 5 days. The storage stability was evaluated by comparing the amount of OPA bis-DNPH remaining in the stored sample with the amount of OPA bis-DNPH in the samples analyzed immediately after preparation.

To determine the yield of the OPA to OPA bis-DNPH reaction in the extraction solution, solutions with 2.55-509 ng/mL OPA were prepared. The reaction yield was determined by comparing the OPA bis-DNPH peak area in the extraction added of OPA solutions with those of the OPA bis-DNPH standard solutions. A calibration curve was constructed by analyzing OPA bis-DNPH standard solutions of 0.05-100 ng/mL. From the calibration curve, the instrumental limit of detection (LOD) and the limit of quantification (LOQ) were defined as 3 and 10 times, respectively, the standard deviation (n = 5) of the peak area of the lowest concentration standard (0.05 ng/mL).

### 3 | RESULTS AND DISCUSSION

#### 3.1 | HPLC-MSMS analytical conditions and sample preparation procedure

A typical HPLC-MSMS chromatogram and ESI mass spectra for a solution extracted from a DNPH-silica cartridge spiked with a solution containing 509 ng OPA are shown in Figure 1. This chromatogram and mass spectrum agreed with those obtained for the OPA bis-DNPH standard solution. Analysis of mass spectra showed a precursor ion peak (Figure 1B) and product ion peak (Figure 1C) at m/z 493 and m/z 182, respectively. The peak at m/z 493 corresponded to deprotonated

![Figure 1](image_url)

**FIGURE 1** A, MRM (m/z 493 > 182) chromatogram of OPA bis-DNPH in standard solution. B, ESI mass spectrum of OPA bis-DNPH (precursor ion). C, MSMS spectrum of OPA bis-DNPH (product ion) and molecular ion structure.
OPA bis-DNPH, and the peak at $m/z$ 182 originated from DNPH.

Our previous study showed that the use of OPA bis-DNPH was suitable for obtaining reliable results via HPLC analysis. Furthermore, we found that the production of OPA bis-DNPH depended on the reaction time and the phosphoric acid concentration in the sample solution, and determined the optimal sample preparation procedure. Using our procedure, the reaction yield from OPA to OPA bis-DNPH in the extraction solution was 91%-104% at OPA concentrations of 2.55-509 ng/mL. Tucker reported that with the addition of excess DNPH to the DNPH-silica cartridge, 100% recovery of OPA was realized after a storage period of 68 hours. This result suggests that the acid (DNPH) in the DNPH-silica cartridge affects the recovery of OPA, but that the amount of excess DNPH was not sufficient to form OPA bis-DNPH. Therefore, the discrepancy between Tucker's results and our results may be due to differences in the amount of excess DNPH or acid in the DNPH-silica cartridges.

### 3.2 Recovery from spiked DNPH-silica cartridges

In the breakthrough test, no OPA was detected in the second DNPH-silica cartridge. Therefore, subsequent sampling was carried out without using a backup DNPH-silica cartridge.

The recoveries from the OPA spiked DNPH-silica cartridges are shown in Table 1. The overall recoveries and the relative standard deviations were 93.1%-100.1% and 1.5%-10.8%, respectively. These results show that this method has excellent recovery and repeatability.

| Spiked amount (ng) | Recovery (%) | RSD (%) |
|-------------------|-------------|---------|
| 0.509             | 100.1 ± 10.8| 10.8    |
| 12.7              | 93.1 ± 9.9  | 10.6    |
| 50.9              | 93.6 ± 1.4  | 1.5     |
| 509               | 98.8 ± 2.0  | 2.0     |

Abbreviation: RSD, Relative standard deviation.

### 3.3 Storage stability of samples

After 5 days of storage, the mean recoveries and standard deviations from the spiked DNPH-silica cartridge were 95% ± 6.6% at 12.9 ng and 99% ± 1.9% at 518 ng. These results indicate that OPA can be stored on a DNPH-silica cartridge for at least 5 days in a refrigerator.

### 3.4 Applicability to measurements at the ceiling exposure concentration

The calibration curve exhibited linearity in the range 0.05-100 ng/mL with a correlation coefficient of $\geq 0.999$. The LOD and LOQ were 0.010 and 0.033 ng/mL (corresponding to 0.050 and 0.165 ng/sample), respectively. These values are much smaller than those of the previous methods, for which LODs of 0.02 and 0.016 µg/sample have been reported.

The ACGIH defines the sampling time for TLV-C as follows: If instantaneous measurements are not available, sampling should be conducted for the minimum period of time sufficient to detect exposures at or above the ceiling value. The minimum sampling time required for the proposed method to measure the ACGIH TLV-C of 0.1 ppb is only 18 seconds (corresponding to a sampling volume of 300 mL at 25°C and 1 atm) based on the LOQ. This time is short enough to measure the ceiling exposure concentration. The sampling times for short-term exposure monitoring (15 minutes), working environment measurements (10 minutes), or the Occupational Exposure Limit-Ceiling as defined by the JSOH (5 minutes or less) are considerably longer than the minimum sampling time required in our proposed method.

Therefore, the proposed method could be adapted to these sampling protocols.

To show the practical applicability of the proposed method, we measured the ceiling exposure concentration of a worker handling disinfectants containing OPA. When the worker poured the disinfectant into the washer for cleaning endoscopes, the measurement was performed with a sampling time of 30 seconds. An OPA concentration of 1.43 ppb was determined.

### 4 CONCLUSION

We developed and validated a highly sensitive method for determination of OPA at the ceiling exposure concentration based on sampling using a DNPH-silica cartridge and HPLC-MSMS analysis. Because the proposed method enabled the determination of an OPA concentration equivalent to the TLV-C of 0.1 ppb proposed by the ACGIH with a sampling time of 18 seconds (corresponding to a sampling volume of 300 mL at 25°C and 1 atm), it will be useful for estimating worker exposure to OPA.

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### DISCLOSURE

Approval of the research protocol: N/A. Informed consent: N/A. Registry and the registration no. of the study/trial: N/A. Animal studies: N/A. Conflict of Interest: N/A.
AUTHOR CONTRIBUTIONS
SY and AT designed the research; SY, TI, and HO collected the data; SY and MI analyzed the data; SY wrote the manuscript; MI and HH led the writing.

ORCID
Shinobu Yamamoto https://orcid.org/0000-0002-0523-1794

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