Development of a method for monitoring personal exposure to benzyl violet 4B and direct blue 15 in workplace air

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Abstract: Objectives: The purpose of this research was to develop a method for monitoring personal exposure to benzyl violet 4B (BV) and direct blue 15 (DB) in workplace air for risk assessment. Methods: We evaluated the utility of the proposed method by examining the following: recovery; method limit of quantification; reproducibility; and storage stability of the samples. Results: An air sampling cassette containing a glass fiber filter was chosen as the sampler. BV and DB were extracted from the sampler filters with a solution of water and methanol (7:3, v/v) and then analyzed by a high-performance liquid chromatograph equipped with a photo-diode array detector. The overall recoveries from spiked samplers were 94-102% and 94-99% for BV and DB, respectively. The recovery after seven days of storage at 4°C exceeded 95%. The method limits of quantification were 0.250 and 1.25 μg/sample for BV and DB, respectively. The relative standard deviations, which represent the overall reproducibility defined as precision, were 0.6-4.1% and 0.8-2.9% for BV and DB, respectively. Conclusions: The proposed method enables 4 h personal exposure monitoring of BV and DB at concentrations of 1-2,000 μg/m³ for BV and 5-2,000 μg/m³ for DB, with a 240 l sampling. Thus, the proposed method is useful for estimating worker exposure to BV and DB.

Introduction

Benzyl violet 4B (BV) and direct blue 15 (DB) were listed as target chemicals in a project on workplace risk assessment carried out by the Ministry of Health, Labour and Welfare (MHLW) of Japan in 2015 because they have been classified as Group 2B (possibly carcinogenic to humans) compounds by the International Agency for Research on Cancer and by the Japan Society for Occupational Health (JSOH). Occupational exposure limits for BV and DB have not been proposed by the JSOH or by the American Conference of Governmental Industrial Hygienists.

To our knowledge, a method for monitoring personal exposure to BV or DB in workplace air has not been reported. The aim of the present study was to develop and validate a personal exposure monitoring method for BV and DB in workplace air with the sampling capacity and sensitivity required for the MHLW exposure survey.

Materials and Methods

Materials

BV and DB were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Acetonitrile, methanol, ammonium dihydrogen phosphate and disodium hydrogen phosphate were of analytical grade or better (for high-performance liquid chromatography). An air sampling cassette (Catalog No. 225-3LF, SKC Inc., Eighty Four, PA, USA) containing a glass fiber filter (37 mm in diameter, Catalog No. AP 2004200, Merck Millipore Ltd., Cork, Ireland) was used.
as the sampler. Sampling was performed by drawing air through the sampler using a SKC Air Check 2000 (SKC Inc., Eighty Four, PA, USA). A mixture of water and methanol (7:3, v/v) was used as the extraction solution. Mixed standard working solutions of BV and DB were prepared in the extraction solution.

**Instruments**

The high-performance liquid chromatograph (HPLC) system used a Shimadzu (Kyoto, Japan) Prominence UFLC equipped with a SPD-M20A photo-diode array (PDA) detector. Separation was achieved using an Inert-Sustain C18 (150 mm × 4.6 mm I.D., 5 μm; GL Sciences Inc., Tokyo, Japan) with a flow rate of 1.0 mL/min at 40°C. The mobile phase was composed of: (A) a 10 mM phosphate buffer containing 5 mM ammonium dihydrogen phosphate and 5 mM disodium hydrogen phosphate; and (B) acetonitrile. The gradient elution program was as follows: 0-20 min, 15-85% B; and 20-30 min, 15% B. The PDA acquisition wavelength was set in the range of 240-800 nm, and the detection wavelengths were 592 nm and 620 nm for BV and DB, respectively.

**Sample preparation**

After sampling, the filter was placed in a glass test tube. Extraction solution (5 mL) was added and the tube was shaken for 5 min, followed by centrifugation at 3,000 rpm for 10 min. The supernatant was then filtered utilizing a DISMIC-13 HP020AN (Advantec Toyo Kaisha, Ltd., Tokyo, Japan). A 10 μl aliquot of the sample solution was injected into the HPLC-PDA.

**Method Validation**

The proposed method was validated in accordance with MHLW guidelines7. A 25 μl aliquot of the mixed standard solution was spiked onto the filter of a sampler. Simultaneously, room air (temperature, 18.6-22.9°C; relative humidity, 29-49%) was drawn through the samplers at a flow rate of 1 l/min for 240 min.

For the recovery test, the spiked amounts ranged from 0.250 to 500 μg for BV and from 1.25 to 500 μg for DB, in a sampling volume of 240 l; these amounts corresponded to air concentrations of approximately 1-2,000 μg/m³ for BV and 5-2,000 μg/m³ for DB. For storage stability tests, different amounts (0.250, 1.25, 25.0 and 500 μg for BV and 1.25, 25.0 and 500 μg for DB) were spiked onto the filter in a sampling volume of 240 l; these amounts corresponded to air concentrations of approximately 1, 5, 100 and 2,000 μg/m³ for BV and 5, 100 and 2,000 μg/m³ for DB. Air was drawn through the spiked samplers, which were then sealed and stored at 4°C for seven days.

**Results and Discussion**

**Selection of sampler and extraction solution**

We used a glass fiber filter as a sampler because it is presumed that BV and DB are solids with very low vapor pressures at ambient temperature. Therefore, they exist as aerosols in the workplace air. We next explored a suitable solvent to extract BV and DB from the glass fiber filter after sampling. Although BV and DB were predominantly insoluble in acetone, acetonitrile, methanol and ethanol, they both were highly water soluble. However, the solutions that were dissolved in water were highly viscous and contained numerous air bubbles that persisted in the solution. Therefore, if water is used as an extraction solution, it may be difficult to obtain an accurate quantitative analysis of BV and DB levels. To overcome this problem, we examined the utility of a mixture of water and methanol to be used as an extraction solution. A low viscosity solution lacking air bubbles was obtained by mixing water and methanol at a ratio of 7:3 (v/v). Extraction efficiencies obtained with this extraction solution from glass fiber filters spiked with BV and DB (each containing 500 μg/sample) were 100±0.9% (mean±standard deviation, n = 5) and 99±0.7%, respectively. Therefore, we utilized this mixed solution as the extraction solution.

**Optimization of the HPLC analytical conditions**

The HPLC-PDA, which is commonly used in many laboratories, was used in this research because the MHLW exposure survey is conducted by several research institutes. To determine the optimum HPLC analytical conditions for BV and DB, we evaluated several reported HPLC analytical conditions for colorants8. We adapted the analytical conditions of the ISO 17234 method™ because it generated both efficient separation and enhanced sensitivity (Fig. 1). Under this analytical condition, no peaks were observed on chromatograms of a solution extracted from a glass fiber filter (Fig. 1[C] and [D]). In contrast, several peaks were observed on chromatograms of a solution extracted from a glass fiber filter spiked with BV and DB (Fig. 1[A] and [B]). These peaks were presumed to be derived from analogs of BV and DB since many analogs are generally contained in the commercially available grades of BV and DB. Therefore, the largest peak on the chromatogram from each standard solution of BV and DB was used as the quantification peak. Absorption spectra of the quantification peaks of BV and DB showed maximum absorption at approximately 592 nm and 620 nm, respectively. These absorption spectra agreed with those from the standard solutions. Therefore, 592 nm and 620 nm were used as the detection wavelengths for BV and DB, respectively.
Fig. 1. Chromatograms of a solution extracted from a glass fiber filter spiked with a mixed standard solution containing 25.0 μg of benzyl violet 4B (BV) and 25.0 μg of direct blue 15 (DB) at (A) 592 nm and (B) 620 nm. Chromatograms of a solution extracted from a blank glass fiber filter at (C) 592 nm and (D) 620 nm.

Table 1. Recovery of BV and DB from spiked sampler

| Spiked amount (μg) | BV Recover a (%) | BV RSD b (%) | DB Recover a (%) | DB RSD b (%) |
|-------------------|-----------------|--------------|-----------------|--------------|
| 0.250             | 94 ± 3.8        | 4.1          | –               | –            |
| 1.25              | 97 ± 1.6        | 1.6          | 94 ± 2.8        | 2.9          |
| 5.00              | 100 ± 0.6       | 0.6          | 95 ± 0.8        | 0.9          |
| 25.0              | 102 ± 0.7       | 0.7          | 99 ± 0.8        | 0.8          |
| 250               | 100 ± 0.9       | 0.9          | 94 ± 0.8        | 0.8          |
| 500               | 99 ± 1.3        | 1.3          | 97 ± 1.3        | 1.4          |

Recovery of the glass fiber filter

The minimum sampling capacity required for the MHLW exposure survey is 240 l (1 l/min, 240 min), and this was evaluated based on the results of the recovery test. The overall recoveries from spiked samplers were 94-102% and 94-99% for BV and DB, respectively (Table 1). Therefore, the glass fiber filter is suitable as a sampler for the MHLW exposure survey of BV and DB.

Storage stability of samples

Storage stabilities were evaluated by comparing the amounts of BV and DB remaining in stored samples with the amounts of BV and DB in the samples analyzed immediately after preparation. After seven days storage, the recoveries from all the spiked samplers exceeded 95%, indicating that BV and DB on a glass fiber filter can be stored for at least seven days at 4°C.

Method limit of quantification and reproducibility

The calibration curves for BV and DB exhibited linearity in the ranges of 0.0500-100 μg/ml and 0.250-100 μg/ml, respectively, with correlation coefficients greater than 0.999. From the calibration curves, the instrumental limit of quantification, defined as 10 times the standard deviation (n = 5) of the peak area of the lowest standard, was 0.153 μg/sample for BV and 0.798 μg/sample for DB. From the results of the recovery test, the method limit of
quantification, defined as the smallest amounts of BV and DB spiked on a sampler filter that resulted in a recovery of more than 90%, was 0.250 μg/sample for BV and 1.25 μg/sample for DB. Therefore, the measurable air concentration ranges for the proposed method are 1-2,000 μg/m³ for BV and 5-2,000 μg/m³ for DB, with a 4 h sample. The relative standard deviations (RSD) of the overall reproducibility of the proposed method, including sampling and analysis, were 0.6-4.1% and 0.8-2.9% for BV and DB, respectively (Table 1). This range of RSD values indicates that the proposed method has good reproducibility.

Conclusions

The proposed method enables 4 h personal exposure monitoring of BV and DB at concentration ranges of 1-2,000 μg/m³ for BV and 5-2,000 μg/m³ for DB. Thus, this proposed method will be useful for estimating worker exposure to BV and DB. To our knowledge, to date there is no report on the personal exposure levels of workers. The results of the MHLW exposure survey in the future may suggest the necessity for development of a new method with higher sensitivity than this proposed method.

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Conflicts of interest: None declared.

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