Brief Report

Determination Method for p-Phenylazoaniline and 2-methyl-4-(2-tolylazo)aniline in Workplace Air by High-performance Liquid Chromatography

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¹Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, Japan, ²Technical Application Department, GL Sciences Inc., Japan, ³GASTEC Corporation, Japan, ⁴Environmental Health and Safety Division, Miyazaki Plant, Baxter Limited, Japan, ⁵Occupational Health Research and Development Center, Japan Industrial Safety and Health Association, Japan and ⁶National Institute of Occupational Safety and Health, Japan

Abstract: Determination Method for p-Phenylazoaniline and 2-methyl-4-(2-tolylazo)aniline in Workplace Air by High-performance Liquid Chromatography: Akito TAKEUCHI, et al. Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association—Objectives: The purpose of this research was to develop a method for the simultaneous determination of p-phenylazoaniline (also called 4-aminoazobenzene, AAB) and 2-methyl-4-(2-tolylazo)aniline (also called o-aminoazotoluene, AAT) in workplace air for risk assessment. **Methods:** The characteristics of the proposed method, such as recovery, limit of quantitation, reproducibility and storage stability of the samples were examined. **Results:** An air sampling cassette containing two sulfuric acid-treated glass fiber filters was chosen as the sampler. The AAB and AAT were extracted from the sampler filters by methanol and then analyzed by a high-performance liquid chromatograph equipped with a photodiode array detector. The overall recoveries from spiked samplers were 77–98 and 85–98% for AAB and AAT, respectively. The recovery after 5 days of storage in a refrigerator exceeded 96%. The overall limits of quantitation were 5.00 and 2.50 $\mu\text{g}/\text{sample}$ for AAB and AAT, respectively. The relative standard deviations, which represent the overall reproducibility defined as precision, were 0.6–1.8 and 0.5–2.2% for AAB and AAT, respectively. **Conclusions:** The proposed method enables 4-h personal exposure monitoring of AAB and AAT at concentrations of 21 to 2,000 $\mu\text{g}/\text{m}^3$ for AAB and 10 to 2,000 $\mu\text{g}/\text{m}^3$ for AAT, respectively. The proposed method

is useful for estimating worker exposure to AAB and AAT.

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Key words: 2-Methyl-4-(2-tolylazo)aniline, Air sampling method, High-performance liquid chromatography, p-Phenylazoaniline, Workplace air

p-Phenylazoaniline (also called 4-aminoazobenzene, AAB) and 2-methyl-4-(2-tolylazo)aniline (also called o-aminoazotoluene, AAT) were listed as the target chemicals in a project on workplace risk assessment carried out by the Ministry of Health, Labour and Welfare (MHLW) of Japan in 2011¹⁾ because they have been classified as Group 2B (possibly carcinogenic to humans) compounds by the International Agency for Research on Cancer (IARC)²⁾ and by the Japan Society for Occupational Health (JOH)³⁾. Occupational exposure limits for AAB and AAT have not been proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) or by the JOH.

To our knowledge, a determination method for AAB or AAT in workplace air has not been reported. In this paper, we report on the development and validation of a high-performance liquid chromatograph (HPLC) method for the simultaneous determination of AAB and AAT in workplace air.

Materials and Methods

Materials

AAB and AAT were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Acetonitrile, formic acid, ammonium acetate, methanol and sulfuric acid were of analytical grade or better (for high-performance liquid chromatography or super special grade). An

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air sampling cassette (Catalog No. 225-3LF, SKC Inc., Eighty Four, PA, USA) containing two sulfuric acid-treated glass fiber filters (Catalog No. 303, GASTEC Corporation, Kanagawa, Japan) was used as the sampler. Sampling was performed by drawing air through the sampler using an SKC Air Check 2000 (SKC Inc., Eighty Four, PA, USA). For calibration curves, working mixed standard solutions of AAB and AAT were prepared in methanol.

Instruments

The HPLC system used a Shimadzu (Kyoto, Japan) Prominence UFLC equipped with a SPD-M20A photodiode array (PDA) detector. Separation was achieved using a InertSustain C18 HP (100 mm $\times$ 3.0 mm I.D., 3 μ m; GL Sciences Inc., Tokyo, Japan) at a flow rate of 1.0 ml/min and at 40°C. The mobile phase was composed of (A) 10 mM ammonium acetate in 0.1% formic acid and (B) acetonitrile. The gradient elution program was as follows: 0.00–5.50 minutes, 20–90% B; 5.50–7.00 minutes, 90% B; 7.00–7.01 minutes, 90–20% B; and 7.01–10.00 minutes, 20% B. The PDA detector was set to scan from 240 to 800 nm, and 385 nm was used as the detection wavelength.

Sample preparation

After sampling, the front and back sample filters were placed in separate glass test tubes. Methanol (5 ml) was added to each tube, the tubes were shaken for 5 minutes, and they were then centrifuged at 3,000 rpm for 10 minutes. An aliquot of 4 μ l of the samples was injected into the HPLC.

Preparation of spiked samplers for retention efficiency and storage stability tests

Mixed standard solutions of AAB and AAT were dissolved in methanol and then diluted with 0.125 M H₂SO₄ in methanol solution. A 25 μ l of mixed standard solution was spiked onto the front filter of a sampler. Simultaneously, room air (temperature, 21–26°C; relative humidity, 22–32%) was drawn through the samplers at a flow rate of 1 l/min for 240 minutes.

For the retention efficiency test, the spiked amounts were varied from 2.5 to 500 μ g for each of AAB and AAT for a sampling volume of 240 l; these correspond to air concentrations of approximately 10–2,000 μ g/m³. For storage stability tests, three different amounts (5.00, 25.0 and 250 μ g for each of AAB and AAT) were spiked onto the filter for a sampling volume of 240 l; these correspond to air concentrations of approximately 20, 100 and 1,000 μ g/m³. Air was drawn through the spiked samplers, and the samplers were then sealed and stored in a refrigerator (4°C) for 5 days.

Results and Discussion

Choice of sampler and optimization of the HPLC analytical conditions

We adopted a sulfuric acid-treated glass fiber filter as a sampler for two reasons. One reason is that it is presumed that AAB and AAT exist as aerosol in the workplace air considering their physical and chemical properties. The other reason is that AAB and AAT are transformed to the corresponding stable sulfate salts on this filter; this was supported by our preliminary experimental results showing that other (a non-treated glass fiber filter and a solid-phase extraction disk) showed low recoveries.

Several HPLC analytical methods have been reported for the determination of AAB and AAT in products (such as leather and toys)^{4–6}. We adopted the method of BS EN 71–11⁶) because the HPLC analytical condition of this method could separate 16 colorants (including AAB and AAT) that could potentially coexist in the workplace. However, this method requires a run time of 45 minutes. Therefore, we modified it to shorten the analysis time. The HPLC analytical conditions (the choice of column, the composition of the mobile phase, the gradient elution program and the detection wavelength) were optimized to ensure a satisfactory separation for fast analysis. Absorption spectra of AAB and AAT showed maximum absorption at around 385 nm. Therefore, the detection wavelength was set at 385 nm. Although overlap of the peaks of AAT and Disperse Blue 124 was observed in the max plot [Fig. 1 (A)], this interference was not observed at 385 nm [Fig. 1 (B)]. Therefore, at least these colorants will not interfere with the determination of AAB and AAT. The run time of the proposed method was reduced to less than a quarter of that of the original method [Fig. 1 (C)].

Retention efficiency of the acid-treated filter

The sampling capacity required for the MHLW exposure survey was 240 l (1 l/min, 240 minutes), and this was evaluated based on the results of the recovery test. No AAB or AAT was detected on the back filter of any of the spiked samplers. Therefore, the acid-treated filter is suitable as a sampler for the MHLW exposure survey of AAB and AAT. The overall recoveries from spiked samplers were 77–98 and 85–98% for AAB and AAT, respectively (Table 1). We consider these results to be satisfactory.

Storage stability of samples

Storage stabilities were evaluated by comparing the amounts of AAB and AAT remaining in storage samples with the amounts of AAB and AAT in the samples analyzed immediately after preparation. After

Fig. 1. Chromatograms of mixed standard solution and each standard solution for 16 colorants (each approximately 50 $\mu\text{g}/\text{ml}$) at (A) max plot (240–800 nm) and (B) 385 nm and (C) chromatograms of p-phenylazoaniline (also called 4-aminoazobenzene, AAB) and 2-methyl-4-(2-tolylazo)aniline (also called o-aminoazotoluene, AAT) in an extracted solution from the acid-treated filter spiked with AAB and AAT mixed standard solution (each 25.0 $\mu\text{g}/\text{sample}$).

Table 1. Recovery from spiked sampler

Spiked amount (μg)	AAB		AAT	
	Recovery ^a (%)	RSD ^b (%)	Recovery ^a (%)	RSD ^b (%)
2.50	77 $\pm$ 1.4	1.8	85 $\pm$ 1.7	2.0
5.00	82 $\pm$ 0.5	0.6	87 $\pm$ 2.0	2.2
12.5	88 $\pm$ 1.0	1.2	92 $\pm$ 0.9	1.0
25.0	97 $\pm$ 0.7	0.7	98 $\pm$ 0.6	0.6
250	96 $\pm$ 0.5	0.6	96 $\pm$ 0.5	0.5
500	98 $\pm$ 0.6	0.6	98 $\pm$ 0.7	0.7

A 25 μl of p-phenylazoaniline (also called 4-aminoazobenzene, AAB) and 2-methyl-4-(2-tolylazo)aniline (also called o-aminoazotoluene, AAT) mixed standard solution diluted with 0.125 M H_2SO_4 in methanol solution was spiked onto the front filter of a sampler. Simultaneously, room air was drawn through the sampler at a flow rate of 1 l/min for 240 minutes. The spiked amounts correspond to air concentrations of approximately 10–2,000 $\mu\text{g}/\text{m}^3$.

^a Values are expressed as means $\pm$ SD (n=5). ^b Relative standard deviation.

5 days of storage, the recoveries from all the spiked samplers exceeded 96%, indicating that AAB and AAT on an acid-treated filter can be stored for at least 5 days in a refrigerator.

Limit of quantitation and reproducibility

The calibration curves for AAB and AAT exhibited linearity in the ranges of 0.0500–100 $\mu\text{g}/\text{ml}$, respectively, with correlation coefficients of above 0.999. From the calibration curves, the instrumental limit of quantitation (LOQ), defined as 10 times the standard deviation (n=5) of the peak area of the lowest standard, was 0.166 $\mu\text{g}/\text{sample}$ for AAB and 0.251 $\mu\text{g}/\text{sample}$ for AAT. From the results of the recovery test, the overall LOQs were found to be 5.00 and 2.50 $\mu\text{g}/\text{sample}$ for AAB and AAT, respectively. These values were the smallest amounts of AAB and AAT spiked on a sampler filter that resulted in a recovery of more than 80%. Therefore, the measurable air concentration ranges for the proposed method are 21 to 2,000 $\mu\text{g}/\text{m}^3$ for AAB and 10 to 2,000 $\mu\text{g}/\text{m}^3$ for AAT, respectively, with a 4-h sample. The relative standard deviations (RSD) of the overall reproducibility of the proposed method, including sampling

and analysis, were 0.6–1.8 and 0.5–2.2% for AAB and AAT, 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 AAB and AAT at concentrations of 21 to 2,000 $\mu\text{g}/\text{m}^3$ for AAB and 10 to 2,000 $\mu\text{g}/\text{m}^3$ for AAT, respectively, and will be useful for estimating worker exposures to AAB and AAT.

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