

Determination Method of Disperse Blue 1 in Workplace Air

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Received: 19 June 2019 • Revised: 17 July 2019 • Accepted: 14 August 2019

Abstract: The first stage to reduce the workers' exposure to the chemicals is a detailed identification of substances in workplaces. The aim of the study was to develop a method for determining the concentration of carcinogenic Disperse Blue 1 in workplace air.

The method consists of drawing the air that contains Disperse Blue 1 through a cellulose filter, washing out the substance adsorbed on the filter with methanol and analyzing the obtained solution using high performance liquid chromatography with a diode array detector. The determination was carried out in the reverse-phase system (mobile phase: acetonitrile: ammonium acetate solution, 45:55) using an Ultra C18 column.

The measurement range was 0.01 - 0.2 mg/m³ for a 720 L air sample. Limit of detection and limit of quantification were 0.52 ng/mL and 1.6 ng/mL, respectively.

The method could be useful for determining occupational exposure to this substance in the working environment.

INTRODUCTION

Disperse Blue 1 (DB1, CAS 2475-45-8), which belongs to the numerous group of disperse dyes, is mainly used for coloring plastics and textiles[1-4]. Disperse Blue 1 also known as C.I. disperse blue 1, C.I. 64500 or 1,4,5,8-tetraaminoanthraquinone is manufactured from 1,5-diaminoanthraquinone. DB1 has been also used in hair-dye formulations at concentrations not exceeding 1% and also in hair mousse [5-7].

International Agency for Research on Cancer classified DB1 as a potentially carcinogenic to humans[3]. Disperse dyes (DB1 included) are responsible for a great majority of allergic contact dermatitis caused by textile dyes[6,7]. The Regulation EC No 1272[8] has classified DB1 as a substance that may cause cancer (category 1B) and can cause skin irritation, and may also cause an allergic reaction after coming into contact with skin.

Increased consumer awareness, associated with the exposure to carcinogenic and allergenic dyes, has led to the introduction of some legislation, such as the EU Eco-label. This legislation restricts the use of several disperse dyes for dyeing textile products that may come into direct and prolonged contact with human skin. Legislations in major markets around the world restricts the presence of DB1 in finished products. The Commission Decision of 5 June 2014[9] established the ecological criteria for the award of the EU Ecolabel for textile products. DB1 is on the Restricted Substances List for raw materials/finished products among other disperse dyes. The routes of potential human exposure to DB1 are inhalation, ingestion, and dermal contact. So far, there are no established workplace air exposure limits for DB1 in the world. Furthermore, there are no methods of determining the DB1 content in the air of the working environment in literature. Available publications describe analyses of the toxic effects of DB1[10-12] and its determination in water[13,14], and consumer products, i.e. textiles[15-20], toys[21,22], hair colorants[23] based, among others, on the procedures described in standards PN-EN ISO 16373-2[24] and PN-EN 71-11[25].

PN-ISO 16373-2 [24] specifies the analyses used to detect extractable dyestuffs in textile products, in which extraction is performed for all kind of fibres and types of dyestuffs using pyridine/water (1:1). Annexes A and B list allergenic and carcinogenic dyestuffs that can be analysed using this method (DB1 among the 22 dispersion dyes listed).

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In this method, the textile sample is extracted with an aqueous solution of pyridine (1:1) for 35 min at temperature of 100 °C. For calibration stock solutions of the reference substances in range of 1 to 20 mg/L are prepared in pyridine-water 1:1.

The European standard PN-EN 71-11 25] for the safety of toys specifies methods for the analysis of toys and toy material extracts prepared to enable the determination of the content of organic chemicals (flame retardants, colourants, primary aromatic amines, monomers and solvents, wood preservatives, preservatives, plasticizers). Colourants (DB1 among the 16 dispersion dyes) are identified and semi-quantified in extracts of toy materials by liquid chromatography with diode-array detector (DAD). If positive identification is obtained, confirmation can be achieved using liquid chromatography with mass spectrometry detection (LC-MS). The concentration range of calibration solutions of dispersion dyes in ethanol is 1 ÷ 5 mg/l.

Occupational exposure to Disperse Blue 1 may occur in workplaces where the substance is manufactured or used. Over the last 25 years the production of dyes in the United States, Western Europe, and Japan has decreased significantly, while production in Asian countries, particularly in China, India, and South Korea, has increased. The textile industry plays a major role in the economy of Asian countries. China and India are major exporters of dyes, as well as large quantities of important raw material and dye intermediate chemicals[26-29].Therefore, there are no data on the occupational exposure during the production of DB1 in the United States and Western Europe.

In European countries occupational exposure to DB1 concerns workers mainly in laboratories where the substance is used as an analytical standard or as a reagent. Information on the type of carcinogenic or mutagenic chemical substances and dusts occurring in Polish enterprises, including the number of exposed employees, is annually reported to the "Central register of data on exposure to carcinogenic or mutagenic chemical substances, mixtures, agents or technological processes", Nofer Institute of Occupational Medicine, Łódź[30].In the years 2013-2016, exposure of workers to DB1 in Poland was reported by universities and scientific institutes.

The experimental part of the study involved developing anew method for determining the concentration of Disperse Blue 1 at workplaces averaged over the sampling time, which could be applied to assess workers' exposure to this substance.

EXPERIMENTAL

Material and Reagents

The following list contains reagents used in the experiment: Disperse Blue 1(Dr. Ehrenstorfer, Germany), 1,4,5,8-tetraaminoanthraquinone (Sigma-Aldrich, Germany), 1,5-diaminoanthraquinone (Aldrich, Germany), methanol, acetonitrile (Merck, Germany) and ammonium acetate (POCh, Polska). Reagents of HPLC grade were used during the tests. The high purity water was obtained from a Milli-Q purification system (Millipore AS, USA). Stock solutions of Disperse blue 1 were prepared in methanol.

Glass microfiber filters, grade GF/A of diameter 37 mm (Whatman, UK) and cellulose filters of 37 mm diameter (POCH, Poland) were chosen for air sampling. For filtering solution before HPLC analysis nylon syringe filters of diameter 25 mm and pore size 0.45 µm (Alltech, USA) were used. Pipettes, volumetric flasks, 25 mL conical flasks with stoppers, other glassware, and syringes were used as additional equipment.

Apparatus

DB1 was analysed using a liquid chromatograph, Agilent Technologies series 1200 with a G2258-90010 autosampler (Agilent Technologies, Germany) coupled on-line with a diode array detector (DAD). Chromatographic separation was performed on an Ultra C18 column (Restek, USA) (250 mm, 4.6 mm ID, 5 µm particle size), which was connected to a guard-column (4 mm ×10 mm) containing the same coating. For data acquisition and processing, Agilent ChemStation software (Rev. B.03.01.) was used.

A Sartorius TE214S analytical balance (Sartorius Corporation, USA) was used to weigh standard substances, AirChek 2000 pump (SKC, USA) was used for collecting air samples. Mechanical shaker WL-2000 (JWElectronic, Poland) was used for DB1 recovery from filters.

Sample Preparation

The filters to which DB1 was applied were left to dry. Then the DB1 was eluted with 3 mL methanol using a mechanical shaker for 30 minutes. The obtained solutions were filtered before HPLC analysis. Filters were stored in suitable cassettes inside a desiccator.

The sample solution was analyzed by means of high performance liquid chromatography with diode array detector (HPLC-DAD).

Chromatographic Conditions

Determination of Disperse Blue 1 was conducted with an Ultra C18 column with a precolumn. Column temperature was set to 30°C. Mobile phase of acetonitrile/ammonium acetate solution (10 mmol/L) (45/55, v/v) at a flow rate of 0.6 mL/min was applied. The volume of the sample injected onto the column was 5 μ L.

The wavelength of diode array detector was optimized. DAD was used at the wavelength of 240 nm and 615 nm. At an analytical wavelength of 240 nm, there are peaks of 1,5-diaminoanthraquinone and DB1 at the chromatogram (Fig. 1). At an analytical wavelength of 615 nm fewer substances may interfere with the determination of DB1 (Fig. 2). Therefore further testing was performed at the analytical wavelength of 615 nm.

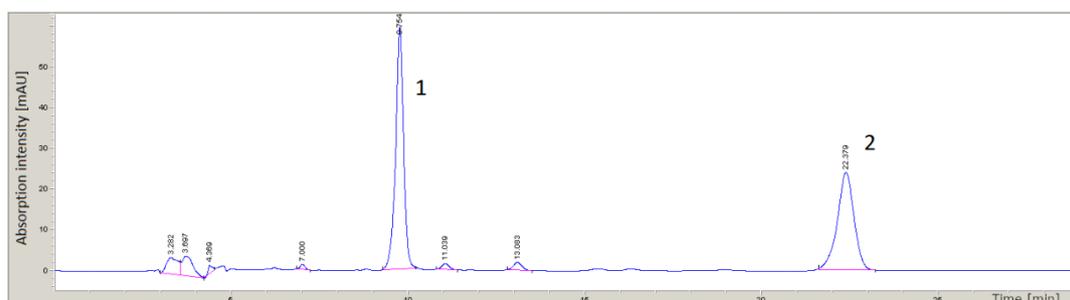


Fig. 1: Chromatogram of the standard solution of DB1 (1) in the presence of 1,5-diaminoanthraquinone (2). HPLC-DAD: column Ultra C18, wavelength of detector 240 nm

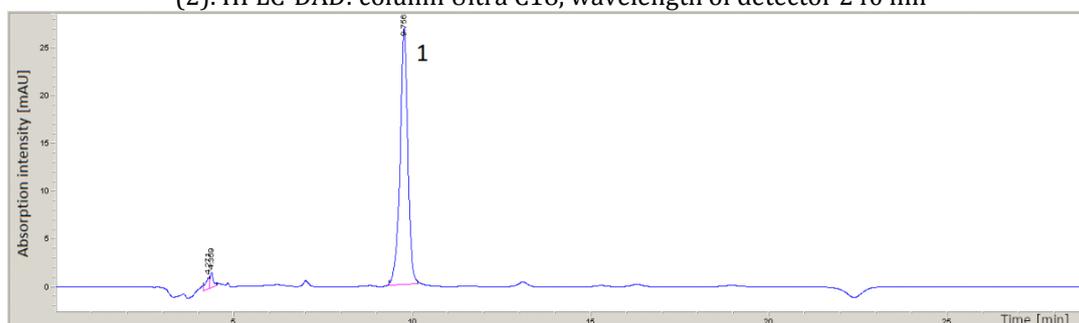


Fig. 2: Chromatogram of the standard solution of DB1 (1) in the presence of 1,5-diaminoanthraquinone (2). HPLC-DAD: column Ultra C18, wavelength of detector 615 nm.

Evaluation of the Overall Method

The process of assessing the measurement procedure and checking its compliance with the established criteria was carried out in accordance with EN 482[31].

The overall approach, including both sampling and analytical methods, was tested by determining the recovery, sensitivity, linearity, precision and expanded uncertainty. Linearity was assessed by linear regression analysis. Sensitivity was evaluated in the same manner as the analytical method. LOD was calculated as the concentration of analyte that gives a response that is significantly different (three standard deviations) from the background response, whereas LOQ (ten standard deviations).

RESULTS AND DISCUSSION

Selection of Filter

There are no data available from literature on air sampling of airborne Disperse Blue1. The method of personal sampling was chosen, where a sampler is worn in the worker's breathing zone during the activities carried out by the worker. Selection of a correct filter material is one of the most important factors, when developing a valid and reliable air sampling method.

The recovery efficiency of DB1 from fibreglass and cellulose filters (diameter 37 mm) was tested as follows: forty microliters of 0.96 mg/mL solution of DB1 were added onto filters (six of each type) and left to dry. After extraction with methanol, solutions from above the filters were analyzed chromatographically.

A higher recovery coefficient was obtained for cellulose filters (0.94) than for fibreglass filters (0.79) (Table 1). For this reason, as well as due to the lower cost of cellulose filters, cellulose filters were used for further tests.

Table 1: The efficiency of fibreglass and cellulose filters for recovery of DB1

Filter material	Average area of DB1 peaks from recovery solutions	Average area of peaks from comparative solutions	Average recovery coefficient
fibreglass	109.7	133.8	0.79
	101.6		
	107.2		
cellulose	122.9		0.94
	125.6		
	128.7		

Air Sampling Conditions

Sampling is performed by drawing a defined volume of air through a cellulose filter using a suitable pump. Breakthrough testing was performed by inserting a cellulose filter (blank) between the cellulose filter spiked with a known amount of DB1 (73.5 µg) and the pump (120 L/h) for 360 min. The test was carried out three times and each sample was injected two times. Breakthrough did not occur (Tab. 2). The results indicate that DB1 is adsorbed on the first cellulose filter. Based on the data collected, 720 L air samples should be collected at a sampling rate of 120 L/h.

Table 2: Results of DB1 adsorption on the cellulose filter. HPLC-DAD: column Ultra C18, wavelength of detector 615 nm

Mass of substance onto filter [µg]	Sampling time [h]	Air flow rate [L/h]	Mean peak area of DB1 in solutions after recovery	
			I filter	II filter
73.5	6	120	302.0	-
73.5	6	120	300.8	-
73.5	6	120	300.1	-

Calibration

For calibration, three series of six standard solutions (2.4, 4.8, 7.2, 12, 24 and 48 µg/mL) of DB1 in methanol were prepared. Analysis of the calibration solutions and measurement of the peak areas of the target substance was performed. Each concentration level was analyzed in duplicate and chromatographic peak areas were fitted by linear least-squares regression. The linear regression for the concentration range from 2.4 to 48 µg/mL was performed achieving correlation coefficients ranging from 0.9999 to 1. Coefficient of variation of the calibration coefficient was 0.63%.

Precision

The precision was studied for eight replicate experiments at three levels (2.4, 24 and 48 µg/mL) of DB1 in methanol. The standard deviations and the coefficients of variation were calculated from the areas of peaks obtained on the chromatograms. The parameters which characterize the precision of calibration of DB1 by HPLC-DAD were acceptable and amounted to 4.34%; 0.69% and 0.99%, respectively.

Recovery

The DB1 standards used to calibrate the HPLC were also used in recovery determination. Spiking was carried out at three appropriate levels (7.2, 72 and 144 µg) on replicate (6) cellulose filters. Recovery of DB1 from spiked cellulose filter was determined by comparing the extracted solutions with the directly injected analyte solutions and ranged from 93 to 96%.

Validation Data

The described method for determination of DB1 in workplace air was validated according to PN-EN 482[31]. The blank sample was used to determine the limit of detection (LOD) and the limit of quantitation (LOQ). Validation data for the determination method of DB1 developed for recommended 720 L of air sample within the dynamic range of 0.01 – 0.2 mg/m³ are presented in Table 3. The limit of quantification was 1.6 ng/mL. This corresponds to 0.0048 µg per sample.

The relative quantification limit was $6.7 \cdot 10^{-6}$ mg/m³ for an air sample volume of 720 liters. The expanded uncertainty for these parameters was 24%.

Table 3: Validation data of the analytical method for determination of DB1

Parameter	Value
Measuring range	0.01 - 0.2 mg/m ³
Volume of air sample	720 L
Calibration curve range	2.4 -48 µg/mL
Limit of detection (LOD)	0.52 ng/mL
Limit of quantification (LOQ)	1.6 ng/mL
Overall precision of the test	5.6%
Expanded uncertainty	24%

CONCLUSION

According to the conducted tests, the method in terms of air sampling and analytical analysis has been established. The concentration of the dye (Disperse Blue 1, DB1) in the air can be measure by drawing the air through a cellulose filter, eluting the DB1 off the filter with methanol, and analyzing using HPLC. Linear calibration curves were obtained within the studied range (2.4 -48 µg/mL) of DB1 concentrations. For air samples of 720 L, the dynamic range will be 0.01 -0.2 mg/m³ of Disperse Blue 1. The results indicate that the sampling and analytical procedure is a reproducible method that ensures consistent quantification of DB1 in the air. The method thus developed can be used for the determination of concentrations of Disperse Blue 1 in workplace air and to assess the occupational risk involved with working with this carcinogen.

ACKNOWLEDGEMENTS

This paper has been based on the results of a research task carried out within the scope of the fourth stage of the National Programme “Improvement of safety and working conditions” supported in 2017–2019 — within the scope of research and development — by the Ministry of Science and Higher Education/National Centre for Research and Development.

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