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RESEARCH ARTICLE

A new method for the determination of 2,2'-dichloro-4,4'-methylenedianiline in workplace air samples by HPLC-DAD

A. Jeżewska^{1,2} and B. Buszewski²

¹Central Institute for Labour Protection—National Research Institute, Warszawa, Poland, and ²Nicolaus Copernicus University, Faculty of Chemistry, Toruń, Poland

Abstract

A new procedure has been developed for the assay of 2,2'-dichloro-4,4'-methylenedianiline (MOCA) using high-performance liquid chromatography with diode array detector. MOCA was sampled from workplace air and derivative before determination using 3,5-dinitrobenzoyl chloride. The determination was carried out in the reverse-phase system (mobile phase: acetonitrile: water) using an Ultra C₁₈ column (Restek, Bellefonte, PA, US). The measurement range was 2–40 µg/m³ for a 100 dm³ air sample. Limit of detection: 7.9 ng/m³ and limit of quantification: 23.8 ng/m³.

Keywords: 4,4'-Methylenebis(2-chloroaniline), MOCA, MBOCA, analytical method, workplace air

Introduction

2,2'-Dichloro-4,4'-methylenedianiline (MOCA) is a crystalline solid obtained in a reaction between 2-chloroaniline and formaldehyde. It is used primarily in plastic industry (polyurethanes, epoxy resins) and in rubber industry (Chen et al., 2005; Liu et al., 2005). The substance may be absorbed into the human body by inhalation, ingestion, and through the skin (Chin et al., 1983; HSDB, 1991; IARC, 1993; ATSDR, 1994; CHEM-BANK, 2000; Sitarek and Lebrecht, 2003).

MOCA may interact with DNA. The formation of DNA adducts in urinary tract epithelial cells and hemoglobin adducts in blood cells was observed in people exposed to the substance in the workplace. Furthermore, an increase in the frequency of sister chromatid exchange and formation of micronuclei in urinary tract epithelial cells and in lymphocytes was proved (Land et al., 1989; McQueen and Williams, 1990; HSDB, 1991; IARC, 1993; ATSDR, 1994; King et al., 1997; CHEM-BANK, 2000; Sitarek and Lebrecht, 2003; Villa and Conso, 2004; SCOEL, 2010). It was classified as a carcinogenic substance (IARC, 1993; Baan et al., 2008; SCOEL, 2010).

As a result of chronic exposure, the compound is likely to cause urinary bladder and hepatic cancer. Long-term

exposure leads to skin redness and inflammation as well as conjunctivitis. Blood changes may be a result of acute poisoning (Vaughan and Kenyon, 1996).

The standard hygiene limit levels (maximum allowable substance air concentrations in the workplace) for MOCA are between 0.005 mg/m³ (Japan, Great Britain) and 0.22 mg/m³ (France, Australia). In Poland, the value of the maximum allowable concentration in workplace air is at a level of 0.02 mg/m³ (Regulation, 2002). Furthermore, it is included in the separate lists of carcinogenic substances (Sweden) without the determination of standard hygiene limit levels, because, it was decided that no safe workplace exposure levels can be established for the substance (CHEM-BANK, 2000; SCOEL, 2010). For this reason, methods of these substances' determination should enable their selective isolation from the workplace air at the lowest possible concentrations levels.

Various procedures for determining MOCA in workplace air have been described. In the method developed by Rappaport and Morales (1979), MOCA-containing air was sampled on a fiberglass filter and a tube filled with silica gel; the analyte was extracted using methanol and assayed using high-performance liquid

chromatography with spectrophotometric detection (HPLC-UV). Limit of detection (LOD) was $3 \mu\text{g}/\text{m}^3$. Purnell and Warwick (1980) determined MOCA using HPLC-UV and HPLC with an electrochemical detector (ED). The air was sampled on a fiberglass filter and MOCA was washed out with methanol from the filter. Limit of quantification (LOQ) when a UV detector was used was $7.5 \mu\text{g}/\text{m}^3$ and for the ED detector it was $0.1 \mu\text{g}/\text{m}^3$. In the method developed by Ebell et al. (1980), samples of MOCA-containing air were taken into glass bubbler filled with a mixture of hydrochloric acid, acetic acid, and water. MOCA was assayed as a derivative of trifluoroacetic acid anhydride using gas chromatography (GC) with an electron capture detector (ECD). The LOD was $0.04 \text{ mg}/\text{m}^3$.

Two methods for MOCA assay in workplace air were developed by the United States Occupational Safety and Health Administration (OSHA). Using OSHA procedure no. 24 (OSHA, 1990), the air was sampled into glass bubblers combined in series which contained hydrochloric acid solution. After the air samples were taken, the solution was neutralized using sodium hydroxide and assayed using an HPLC-UV method. The concentration range which can be determined using the method is $0.062\text{--}0.43 \text{ mg}/\text{m}^3$. In OSHA procedure no. 71 (OSHA, 1989), the air was sampled into filters with sulfuric acid applied. After derivatization with heptafluorobutyric acid anhydride, the MOCA derivative was assayed using GC-ECD technology. The desorption coefficient was 0.93. The LOQ was $0.44 \mu\text{g}/\text{m}^3$.

The procedure provided in the Polish Standard (PN-Z-04295, 2003) involves the deposition of MOCA aerosol onto a polypropylene filter and vapors on Tenax TA, desorption with methanol and analysis of the resulting solutions using a GC-flame ionization detector method. The lowest MOCA air concentration which can be determined using the method is $0.02 \text{ mg}/\text{m}^3$.

This paper discusses a new methodology for MOCA assay in workplace air in which the air is sampled on a filter with sulfuric acid applied. After derivatization with 3,5-dinitrobenzoyl chloride (DNB), MOCA is analyzed in a derivatized form using HPLC with diode detection (HPLC-DAD).

Experimental

Equipment

Throughout the study, an Agilent Technologies (Waldbronn, Germany) series 1200 liquid chromatograph with a diode detector (DAD) in on-line configuration were used. The samples were fed using an automatic injector. ChemStation Software was used for process control, determination and data acquisition. Ultra C_{18} column; dimensions: $(250 \times 4.6 \text{ mm})$ with $\text{dp} = 5 \mu\text{m}$, with precolumn, dimensions: $10 \times 4.0 \text{ mm}$ (Restek, Bellefonte, PA). Gilair 5 (Sensidyne, Clearwater, FL) aspirator for air sampling. Promax 1020 (Heidolph, Schwabach, Germany) mechanic shaker for MOCA recovery from the filter. A water bath (Laboplay, Bytom, Poland) or a

microwave oven (Panasonic, Kusatsu, Japan) for heating the reaction mixture.

Material and reagents

The following reagents were used in the study: MOCA from Riedel-de Haën (Seelze, Germany), DNB (Merck, Darmstadt, Germany), acetonitrile (ACN), methanol, and toluene (J. T. Baker, Deventer, The Netherlands), high-purity water produced by the Milli-Q apparatus (Millipore, Bedford, MA). Sulfuric acid and sodium hydroxide (POCH; Gliwice, Poland). HPLC-grade reagents were used throughout the study.

Materials: fiberglass filters with 37 mm diameter, Whatman GF/A (Maidstone, UK).

Methodology

Air samples which contained MOCA (100 dm^3) were sampled onto two filters with sulfuric acid solution applied, connected in series. Subsequently, each filter was placed in a separate conical flask. Two milliliters of distilled water was used for the recovery of MOCA disulfate deposited on the filter and the filters were left to stand for approximately 1 h. Subsequently, 2 mL of sodium hydroxide solution (concentration of 0.135 mol/L) and 1 mL of toluene was added. The flasks were shaken for 30 min. The solutions from above the filter were transferred into test tubes and left to stand (about 5 min) until equilibrium between the aqueous and toluene phase was reached. Subsequently, 0.5 mL of the toluene layer was taken and transferred into 2 mL vessels to which 20 μL of DNB solution in toluene (concentration: 1 mg/mL) was added. The derivatization reaction was carried out using two procedures: on a water bath (80°C ; 30 min) or in a microwave oven (600 W; $2 \times 2 \text{ min}$). The toluene was evaporated in nitrogen stream and the dry residue was dissolved in 0.5 mL of ACN. The resulting solutions were subjected to chromatographic analysis. The procedure for sample preparation for analysis is shown in Figure 1. The determination was carried out using an HPLC type Ultra C_{18} column with a precolumn. Measurement temperature was 23°C . The mobile phase flow rate was 1 mL/min . ACN:water was used as the mobile phase (70:30 v/v). The volume of injected samples was 20 μL . A DAD detector with an analytical wavelength of $\lambda = 232 \text{ nm}$ was used. In such conditions, MOCA could be determined in the presence of other aromatic amines, such as: aniline, benzidine, 4,4'-methylenedianiline, and 2-chloroaniline (Figure 2).

Results and discussion

MOCA sorption analysis

MOCA sorption from the air was tested using a laboratory system which consisted of a gas pipette, two filters (with sulfuric acid applied) connected in series, suction pump with air volume stream regulated and controlled by a rotameter. Five microliters of MOCA methanolic solution

with a concentration of 2 mg/mL was measured into a gas pipette, which corresponded to a content of 10 µg of the test substance, and 100 dm³ of air were passed at a constant volume stream of 100 dm³/h and 20 dm³/h. During the analysis, the gas pipette was heated using a heating lamp. MOCA was deposited on the fiberglass filter with sulfuric acid applied. The solutions obtained after recovery from the other filter did not contain MOCA. The data are shown in Table 1.

Selection of the derivatization reagent

Amines have alkaline properties and they readily react with inorganic acids whereby the salts form. Therefore, it is possible to recover them from the tested air using

selective sorption onto filters with applied sulfuric acid. The reaction is reversible in the alkaline environment. Taking advantage of this property, the recovery is carried out using sodium hydroxide. In order to gain a better LOQ for MOCA, it was necessary to convert the assayed substance into a derivative. To this end, it was verified whether the following isothiocyanates could be used as the derivatization agents: 1-naphthyl, phenyl, 4-nitrophenyl, allyl and ethyl and also 4-chloro-7-nitrobenzofurazan, 4-toluenesulphonyl chloride, and DNB (Evgen'yev et al., 1995; Sahasrabudhhey et al., 1999; Toyo'oka, 1999; Lindahl et al., 2001; Verdú-Andrés et al., 2001; Nollet, 2006; Dziarkowska et al., 2008). According to literature data, the substances react with

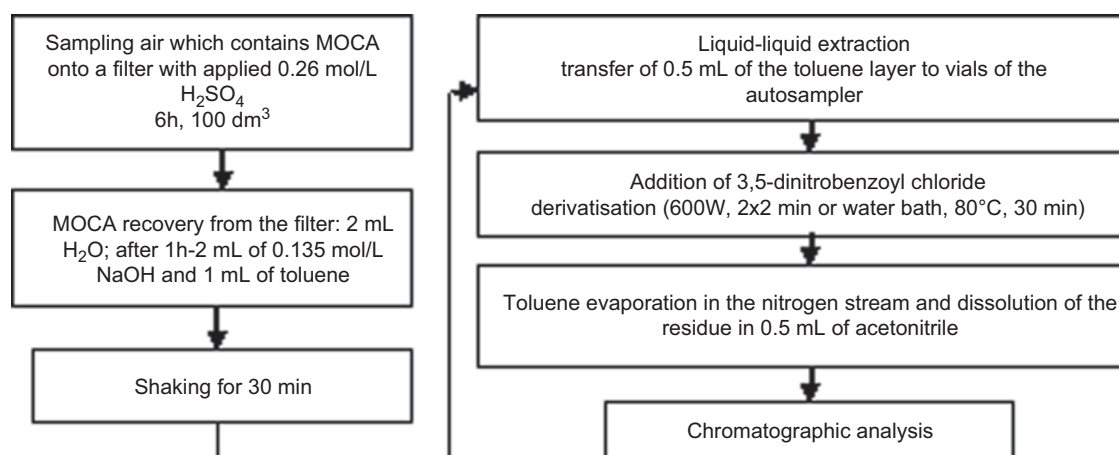


Figure 1. Scheme of the preparation of the analytical sample.

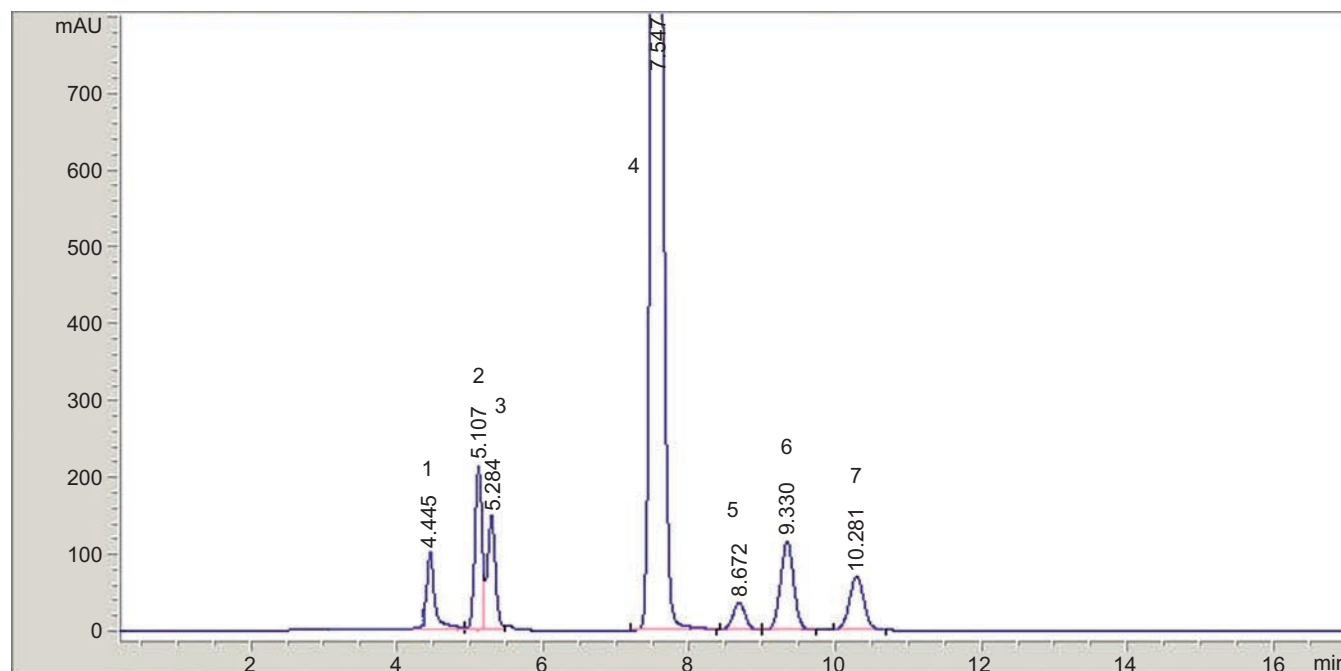


Figure 2. Chromatogram of aromatic amine derivatives obtained following the derivatization of 3,5-dinitrobenzoyl chloride. (1-peak from 3,5-dinitrobenzoyl chloride; 2-aniline derivative; 3-2-chloroaniline derivative; 4-toluene; 5-benzidine derivative; 6-4,4'-methylenedianiline derivative; 7-MOCA derivative). Determination conditions: HPLC-DAD, Ultra C18 (250×4.6 mm, 5 µm) column, column temperature: 23°C; mobile phase: ACN: H₂O, 70:30 (v/v); 1 mL/min. HPLC-DAD, high-performance liquid chromatography with diode detection.

primary amines. The measure of the selection of the derivatization reagent was the potential for increasing assay sensitivity. Therefore, reactions between MOCA and the aforementioned substances in toluene solution were carried out at room temperature and upon heating the reaction mixture to 80°C. The reaction between MOCA and DNB at a concentration range of 0.08–0.8 µg/mL occurred at room temperature after 1 day. The reaction between MOCA (concentration: 0.08 µg/mL and 0.8 µg/mL in toluene) with isothiocyanates, 4-chloro-7-nitrobenzofurazan and 4-toluenesulphonyl chloride did not occur even upon heating to 80°C. DNB was selected for further studies, as it was the only substance that reacted with MOCA resulting in a satisfying DAD detector signal (Figure 3).

The reaction between MOCA and DNB in toluene solution at room temperature ran very slowly. After 1 h of reaction carried out in such conditions, the peak from unreacted MOCA was still seen in the chromatogram (Figure 4). Therefore, the reaction mixture was heated. It was determined as a result of the study that when the reaction mixture is heated on a water bath, the reaction is complete after 30 min at 80°C. The time can be reduced when the derivatization reaction is carried out in microwave radiation. The reaction time can be then reduced to 4 min (2 × 2 min; 600 W) with the results being similar.

Table 1. Example results of MOCA (2,2'-dichloro-4,4'-methylenedianiline) adsorption on the fiberglass filter with sulfuric acid placed on it. The study proved that MOCA is completely retained on filter 1 when sampling air. The control solutions obtained after recovery from filter 2 did not contain the test substance.

Air flow of absorbed air (dm ³ /h)	Approximate substance concentration in the air (µg/m ³)	Area peaks of the MOCA derivative in solutions after recovery (according to the indications of ChemStation Software)	
		Filter 1	Filter 2
100	100	1274.5	No MOCA
20	100	932.1	No MOCA

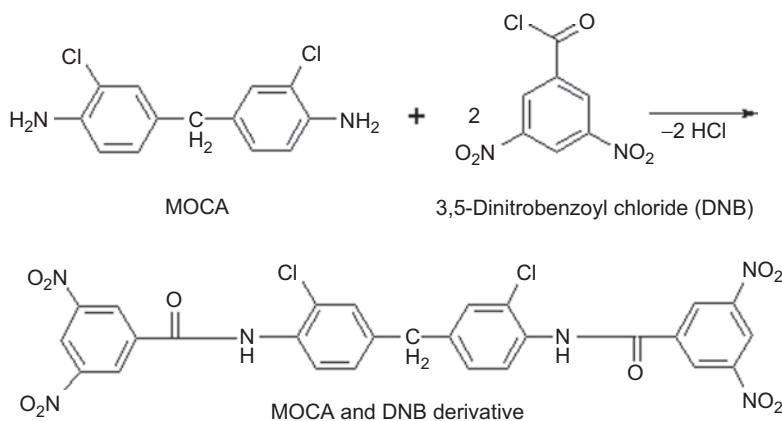


Figure 3. MOCA (2,2'-dichloro-4,4'-methylenedianiline) derivatization reaction.

Validation

Calibration and precision

The assessment of the precision of calibration tests was carried out for six series of solutions. Each series consisted of working solutions with the following concentrations: 0.2 µg/mL, 0.3 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL, and 4 µg/mL of MOCA in toluene which, following the derivatization with DNB (the reaction proceeded on a water bath) and solvent exchange to ACN, was assayed by chromatography as an MOCA derivative (Figure 5, curve A). The linearity of the standard curve is characterized by the correlation coefficient. Correlation coefficient: $R^2 = 0.9997$.

The second curve (Figure 5, curve B) was obtained when 10 µL, 15 µL, 25 µL, 50 µL, 100 µL, and 200 µL of the MOCA standard solution in methanol (concentration: 20 µg/mL) was successively applied on the filters. Subsequently, the scheme shown in Figure 1 was followed; however, the derivatization reaction was carried out on a water bath (80°C, 30 min). The correlation coefficient which characterizes the linearity of the standard curve R^2 is 0.9994.

The third curve (Figure 5, curve C) was obtained when 10 µL, 15 µL, 25 µL, 50 µL, 100 µL, and 200 µL of the MOCA standard solution in methanol (concentration: 23.6 µg/mL) was successively applied on the filters. Subsequently, the scheme shown in Figure 1 was followed. The derivatization reaction was carried out in microwave radiation. The correlation coefficient which characterizes the linearity of the standard curve R^2 is 1. With this calibration procedure (the calibration curve was determined by the application of the test substance on filters) it is not necessary to determine recovery yield, because the filters with MOCA sampled in the workplace are subjected to the same preparation procedure for analysis as filters with the substance applied in the laboratory for the determination of the standard curve. Any possible losses of the substance due to successive stages of sample preparation for analysis are identical for the test sample as well as in the preparation of the standard curve. If so, the degree of recovery is always equal to 1.

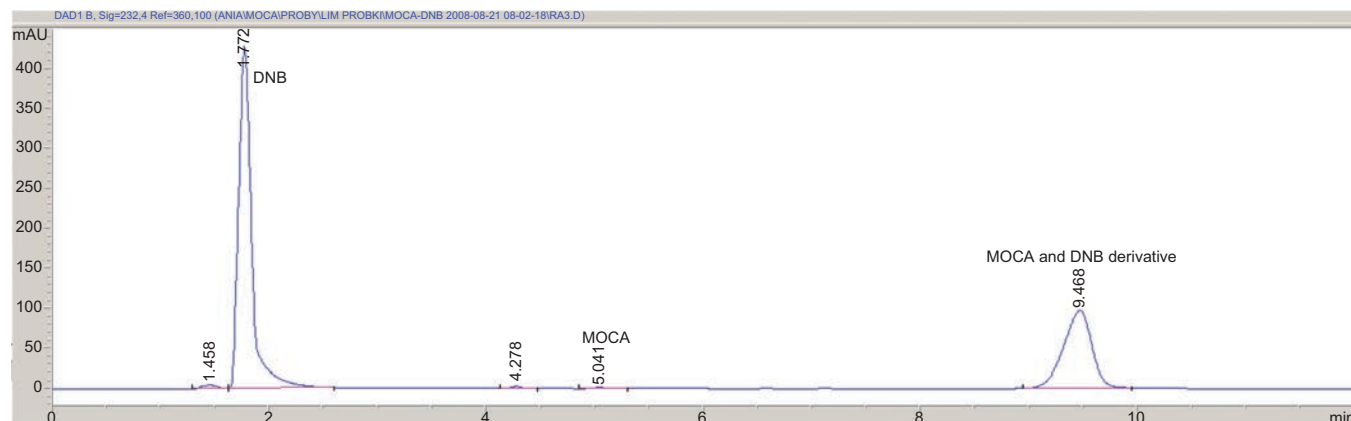


Figure 4. Chromatogram of the MOCA (2,2'-dichloro-4,4'-methylenedianiline) derivative after 1 h of reaction with 3,5-dinitrobenzoyl chloride carried out at room temperature. Determination conditions: HPLC-DAD ($\lambda=232$ nm), Ultra C18 (250 \times 4.6 mm, 5 μ m) column without a precolumn, column temperature: 23°C; mobile phase: ACN: H₂O, 70:30 (v/v); 1 mL/min. HPLC-DAD, high-performance liquid chromatography with diode detection.

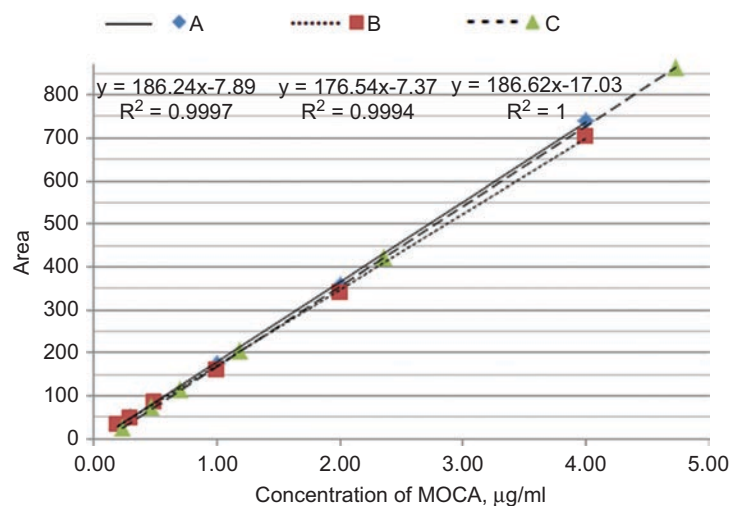


Figure 5. Plot of the relationship between peak area of the MOCA (2,2'-dichloro-4,4'-methylenedianiline) derivative and MOCA concentration in calibration solutions obtained using three methods: (A) from standard solutions (derivatization reaction carried out on a water bath); (B) from filters (derivatization reaction carried out on a water bath); (C) from filters (derivatization reaction carried out in a microwave oven).

When carried out in microwave radiation, the derivatization of the substance recovered from filters leads to lower losses of the substance than when performed on a water bath.

Testing the degree of recovery for three concentrations in the measurement range

In order to determine the degree of MOCA recovery from filters for three concentrations in the measurement range, 15 μ L, 50 μ L, and 150 μ L of the MOCA solution in methanol (concentration: 20 μ g/mL) each was applied onto six filters. The filters were dried. A system was assembled from two filters connected in series (the first filter with the analyte applied and the other protecting filter without the analyte) and 100 dm³ of pure air was passed through the filters at a volume stream of 17 dm³/h. Having sampled the air, each filter was transferred into a separate flask and treated as before. Mean recovery

coefficient $R=100 \pm 3\%$ (Figure 6). The control solutions obtained after recovery from the other filter did not contain the test substance ($R=0$).

The procedure was validated according to European Standard PN-EN 482 (2009).

$$LOD = \frac{3,3 \cdot s_o}{b} \quad (1)$$

The following formula was used for the calculation of LOD, where: b is the slope of the calibration curve, s_o is the standard deviation.

In order to calculate standard deviation (s_o) of the results obtained for a series of blind samples, 10 independent peak surface measurements were conducted at a retention time of the test analyte for three blind samples prepared independently (samples prepared identically as the actual sample, without the analyte). The validation data obtained based on the results of tests are shown in

Table 2. When the recovery process is included in the calibration curve (second method, curve B), the relative value of total uncertainty is reduced. With the analysis carried out according to this method, the reproducibility of results is vital (relative standard deviation was less than 5%).

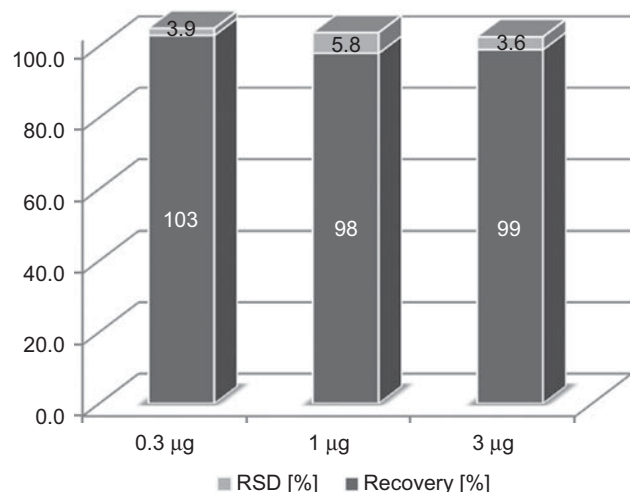


Figure 6. MOCA (2,2'-dichloro-4,4'-methylenedianiline) recovery from filters ($n=6$ for each concentration level).

Table 2. Validation data of the method (A and B) of analytical curve preparation.

Measurement range: 0.2–4 µg/mL (2–40 µg/m³ for 100 dm³ air sample)

Method of analytical curve preparation	(A) from standard solutions	(B) from application onto filters
Limit of detection (LOD)	0.75 ng/mL (7.5 ng/m ³)	0.79 ng/mL (7.9 ng/m ³)
Limit of quantification (LOQ)	2.26 ng/mL (22.6 ng/m ³)	2.38 ng/mL (23.8 ng/m ³)
Analytical curve with formula:	$y = 186.24x - 7.9$	$y = 176.54x - 7.37$
Correlation coefficient (R)	0.9999	0.9997
Analytical coefficient of variation	4.15%	3.69%
Overall precision of the examination	5.61%	5.61%
Relative overall uncertainty	13.13%	11.22%

The total analytical precision (V_c) which includes laboratory precision for the measurement range and sampling technique was determined from the following formula:

$$V_c = \sqrt{V_z^2 + V_p^2}, \quad (2)$$

where: V_p is the precision of the sampling device ($V_p = \pm 5\%$); V_z is the average precision of three levels within the range (0.25 µg/mL, 1.25 µg/mL, and 3.75 µg/mL), calculated from the formula:

$$V_z = \sqrt{\frac{\sum (n_j - 1) \cdot V_i^2}{\sum (n_j - 1)}}, \quad (3)$$

where: n_j , number of repeated samples ($n_j = 8$); V_i , variation coefficient for the concentration level (i.e., 2.89%; 2.83%; 1.73%).

The total analytical precision determined in this way is 5.61%.

Conclusions

As a result of the testing, a sorbent was selected (a filter with applied sulfuric acid) for MOCA absorption from the air. Owing to the application of the filter, it was possible to sample the air in the worker's breathing zone (and thus in line with the principles of individual dosimetry) and, therefore, to obtain the most reliable results for the assessment of workplace exposure.

The derivatization reagent was selected along with the conditions of its reaction with MOCA. A linear standard curve was obtained in the test range (2–40 µg/m³). It was suggested to determine the standard curve, which took into consideration the stage of sample preparation for analysis. This analytical procedure eliminates the stage of recovery coefficient determination; therefore, the time needed for analysis is reduced and less reagents are used. Owing to the derivatization reaction in microwave radiation, better testing results can be obtained and reaction time is reduced (from 30 min to 4 min). Table 3 shows a comparison of characteristic features of the procedure to that developed by OSHA (OSHA, 1990), being most frequently used for the determination of test substance concentration. The developed procedure can be used to assay MOCA

Table 3. Comparison of the two methods of analytical determination of MOCA in workplace air: OSHA Method No 71 and method worked out Central Institute for Labour Protection—National Research Institute (CIOP-PIB).

Method	Work out in CIOP-PIB	OSHA No. 71
Taking samples	Two filters with sulfuric acid	Two filters with sulfuric acid
Volume of air taken for the analysis	100 dm ³	100 dm ³
Recovery of MOCA from the filter and sample preparation of the sample for the analysis	Water, NaOH, toluene Liquid-liquid extraction derivatization using 3,5-dinitrobenzoyl chloride	Water, NaOH, toluene Liquid-liquid extraction derivatization using heptafluorobutyric acid anhydride
Analytical technique	HPLC-DAD	GC-ECD
Measurement range	2–40 µg/m ³	109–436 µg/m ³
Limit of quantification (LOQ)	23 ng/m ³	440 ng/m ³

air concentrations 19 times lower than in the OSHA procedure.

Declaration of interest

The authors report no conflicts of interest.

References

- ATSDR. 1994. Toxicological profile for 4,4'-methylenebis(2-chloroaniline) MBOCA. U.S. Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. Atlanta, Georgia.
- Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Coglian V. 2008. Carcinogenicity of some aromatic amines, organic dyes, and related exposures. *Lancet Oncol* 9:322–323.
- CHEM-BANK. 2000. (CD-ROM), London: Silver Platter Information.
- Chen HI, Liou SH, Loh CH, Uang SN, Yu YC, Shih TS. 2005. Bladder cancer screening and monitoring of 4,4'-methylenebis(2-chloroaniline) exposure among workers in Taiwan. *Urology* 66:305–310.
- Chin B, Tobes MC, Han SS. 1983. Absorption of 4,4'-methylenebis[2-chloroaniline] by human skin. *Environ Res* 32:167–178.
- Dziarkowska K, Jönsson JA, Wiczorek PP. 2008. Single hollow fiber SLM extraction of polyamines followed by tosyl chloride derivatization and HPLC determination. *Anal Chim Acta* 606:184–193.
- Ebell GF, Fleming DE, Genovese JH, Taylor GA. 1980. Simultaneous determination of 2,4- and 2,6-diisocyanatotoluene (TDI) and 3,3'-dichloro-4,4'-diaminodiphenylmethane (MOCA) in air. *Ann Occup Hyg* 23:185–188.
- Evgen'yev MI, Garmonov SY, Evgen'yeva II, Budnikov HC. 1995. Determination of hydrazine derivatives by flow-injection analysis with spectrophotometric detection. *Talanta* 42:1465–1469.
- HSDB. 1991. 4,4'-Methylenebis(2-chloroaniline). Hazardous Substances Data Bank. U.S. National Library of Medicine. Toxicology Information Program, Bethesda, Maryland.
- IARCancer. 1993. Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. In: *Monographs on the Evaluation of the Carcinogenic Risks to Humans*. Volume 57. Lyon, France: International Agency for Research on Cancer.
- King CM, Land SJ, Jones RF, Debiec-Rychter M, Lee MS, Wang CY. 1997. Role of acetyltransferases in the metabolism and carcinogenicity of aromatic amines. *Mutat Res* 376:123–128.
- Land SJ, Zukowski K, Lee MS, Debiec-Rychter M, King CM, Wang CY. 1989. Metabolism of aromatic amines: relationships of N-acetylation, O-acetylation, N,O-acetyltransfer and deacetylation in human liver and urinary bladder. *Carcinogenesis* 10:727–731.
- Lindahl R, Wästerby A, Levin JO. 2001. Determination of morpholine in air by derivatisation with 1-naphthylisothiocyanate and HPLC analysis. *Analyst* 126:152–154.
- Liu CS, Liou SH, Loh CH, Yu YC, Uang SN, Shih TS, Chen HI. 2005. Occupational bladder cancer in a 4,4'-methylenebis(2-chloroaniline) (MBOCA)-exposed worker. *Environ Health Perspect* 113:771–774.
- McQueen CA, Williams GM. 1990. Review of the genotoxicity and carcinogenicity of 4,4'-methylene-dianiline and 4,4'-methylene-bis-2-chloroaniline. *Mutat Res* 239:133–142.
- Nollet LML. 2006. *Chromatographic Analysis of the Environment*. Chromatographic Science Series. 3rd edn. Volume 93. CRC Press, Lincoln, NE.
- OSHA Method No 24. 1990. 4,4'-Methylenebis(o-chloroaniline) [MOCA], U.S. Department of Labor, Occupational Safety and Health Administration, Organic Methods Evaluation Branch, OSHA Analytical Laboratory. 2nd edn. Salt Lake City, Utah.
- OSHA Method No 71. 1989. o-Dianisidine, 4,4'-Methylenebis(2-chloroaniline), o-Tolidine. U.S. Department of Labor. Occupational Safety and Health Administration, Organic Methods Evaluation Branch, OSHA Analytical Methods Manual. Salt Lake City, Utah.
- PN-EN 482. 2009. Workplace atmospheres. General requirements for the performance of procedures for the measurement of chemical agents.
- PN-Z-04295. 2003. Protection of air cleanliness. Assay of 2,2'-dichloro-4,4'-methylenedianiline in the workplace using gas chromatography.
- Purnell CJ, Warwick CJ. 1980. Application of electrochemical detection to the measurement of 4,4'-methylenebis(2-chloroaniline) and 2-chloroaniline concentrations in air by high-performance liquid chromatography. *Analyst* 105:861–867.
- Rappaport SM, Morales R. 1979. Air-sampling and analytical method for 4,4'-methylenebis(2-chloroaniline). *Anal Chem* 51:19–23.
- Regulation of the Minister of Labour and Social Policy of Poland of 29 November 2002 on the maximum allowable concentrations and levels of agents harmful for the health in the workplace. *Journal of Laws* No. 217, Item 1833 as amended.
- Sahasrabudhhey B, Jain A, Verma KK. 1999. Determination of ammonia and aliphatic amines in environmental aqueous samples utilizing pre-column derivatization to their phenylthioureas and high performance liquid chromatography. *Analyst* 124:1017–1021.
- SCOEL. 2010. Recommendation from the Scientific Committee on Occupational Exposure Limits for 4,4'-Methylene-bis-(2-chloroaniline) [MOCA], SCOEL/SUM/174, Luxembourg, June 2010.
- Sitarek K, Lebrecht G. 2003. 2,2'-Dichloro-4,4'-methylenedianiline. Documentation of maximum admissible values for occupational exposure. *Podstawy i Metody Oceny Środowiska Pracy* 36:135–156.
- Toyo'oka T. 1999. *Modern Derivatization Methods for Separation Sciences*. New York: John Wiley and sons.
- Vaughan GT, Kenyon RS. 1996. Monitoring for occupational exposure to 4,4'-methylenebis(2-chloroaniline) by gas chromatographic-mass spectrometric analysis of haemoglobin adducts, blood, plasma and urine. *J Chromatogr B Biomed Appl* 678:197–204.
- Verdú-Andrés J, Campíns-Falcó P, Herráez-Hernández R. 2001. Determination of aliphatic amines in water by liquid chromatography using solid-phase extraction cartridges for preconcentration and derivatization. *Analyst* 126:1683–1688.
- Villa AF, Conso F. 2004. Amines aromatiques. EMC—Toxicologie-Pathologie 1:161–177.