Determination of Isocyanates in Workplace Atmosphere by HPLC

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Determination of diisocyanates in the laboratories of the Authorities of Public Health was described and verified. Air samples collected in the breathing zone of workers exposed to diisocyanates were analyzed in an automotive industry. Diisocyanates (2,4-toluene diisocyanate, 2,6-toluene diisocyanate, 4,4'-methylenediphenyl isocyanate, and 1,6-hexamethylene diisocyanate) were measured by HPLC and the validation characteristics were determined. Diisocyanate exposure was monitored in the workplace atmosphere of 25 workers. Air samples from the breathing zone were collected and analyzed by HPLC after extraction. The results were compared to the occupational exposure limit. Reliability of the method was confirmed by validation characteristics for the diisocyanates. The repeatability of the method ranged from 2.98 to 4.51%, the calculated relative standard uncertainty was 11 - 12% for the parameters, and the recovery was between 99 and 103%. The low LOD and LOQ ensured the determination of the diisocyanates in the harmful concentration. Monitoring of diisocyanate and 2,6-toluene diisocyanate were present in the concentration range from 6.3 to 13.2 μ g m³. The 1,6-hexamethylene diisocyanate was found in all cases below the limit of detection and the 4,4'-methylenediphenyl isocyanate was found only at two workplaces (between 8.3 and 44.8 μ g m³). The HPLC method was found to be appropriate for the determination of diisocyanates. Applying the method for the determination of diisocyanate level did not exceed the occupational exposure limit set for average exposure.

Keywords: Monitoring of Workplace Atmosphere, Diisocyanates, HPLC, Method Validation

The work is focused on the monitoring and analysis of the most frequent diisocyanates (DIs) in workplace atmosphere. Isocyanates are organic compounds having one or more highly reactive isocyanate groups (-N=C=0)bonded to an aromatic or aliphatic hydrocarbon chain. Diisocyanates are compounds with two isocyanate groups, which have reactivity to water and various organic compounds, for instance with amines, producing ureides (-NHCONH-), or with alcohols producing ureates (-NHCOO-). The most significant reaction used in the industry is with polyhydroxy alcohols producing polyurethane (PU) polymers. PU products are widely used in the furniture industry (mattresses), in automotive industry (seats, coating compositions), in building industry (insulation and coating compositions), in textile and shoe industry (glues), electrical and refrigerating engineering, etc. [1].

Monoisocyanates (part of pesticides), diisocyanates and oligoisocyanates are often used in practice. In 2000, the world trade of diisocyanates (DIs) was 4.4 million tons, including methylene diphenyl diisocyanate (61.3%), toluene diisocyanates (34.1%), hexamethylene diisocyanate and isophorone diisocyanate (3.4%), and others (1.2%) [2, 3].

Occupational exposure to DIs can occur during the production and raw material processing, as well as in further handling with the final products, especially in the activities joined with thermal degradation (cutting and welding operations).

Diisocyanates are major allergens at low concentration levels and significantly contribute to the development of occupational asthma [4-6]. Suitable serologic tests for the determination of specific antibodies in clinical diagnosis of diisocyanate asthma have been developed by allergologists and immunologists to obtain information on human exposure to these compounds [7-9].

The inhalation was suggested as the principial exposure route for isocyanates [10]. The other main intoxication routes are via contact with skin, and mucous in eyes. DIs can cause inflamation of eyes and skin, and, therefore, are considered as strong sensibilisers. Exposure can lead to strong allergic reactions on skin or problems with respiratory organs-cough, dyspnoea or asthma. Nakashima et al. summarized effects of occupational exposure to DIs. [11]. Animal and human studies provided the evidence of genotoxic, mutagenic and carcinogenic effects of toluene diisocyanates and methylenediphenyl diisocyanate. Generally, DIs are known to have toxic, carcinogenic and mutagenic effects. The main routes of exposure by isocyanates are inhalation of vapors or aerosols during spraying or skin contact during handling of liquid isocyanates.

The role of health service officers and medical doctors is to perform supervision on the workplaces, where risk of

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exposure to chemicals may occur in order to prevent health damage [12-14].

The most commonly used diisocyanates in industry are: 1,6-hexamethylenediisocyanate (1,6-HDI); 2,4-toluene diisocyanate (2,4-TDI); 2,6-toluene diisocyanate (2,6-TDI); and 4,4⁻-methylenediphenyl diisocyanate (4,4⁻-MDI) (fig.1).

1,6-HDI is a homopolymer, used as an activator that improves technical features of polyurethane coatings such as durability, color stability, resistance to abrasion, chemicals, and weather influence [15]. Exposure to hexamethylenediisocyanate homopolymers is in positive relation with occurrence of asthmatic reactions [16]. Exposure of rats to hexamethylenediisocyanate in laboratory conditions caused respiratory tract irritation [15].

Mixture of 2,4- and 2,6-toluene diisocyanate is available as an important industrial chemical in the production of polyurethane polymers. Toluene diisocyanates may cause irritation of the mucous membranes, asthma and progressive impairment of pulmonary function. Animal studies showed that exposure to toluene diisocyanates increased risk of cancer [17].

4,4⁻-methylenediphenyl diisocyanate is mainly used in the industrial production of rigid polyurethane foams and in the fields of coatings, adhesives, sealants and elastomers such as paints, adhesives, weather-resistant sealing materials and footwear. 4,4⁻-MDI is an irritant and a sensitizer; exposure by inhalation produces asthma among workers. Human and animal studies showed strong association among exposure to 4,4⁻-MDI and risk of several types of cancer [18].

The occupational exposure limits for the gases, vapors and aerosols (OEL) in working environment for diisocyanates in the Slovak Republic (Government regulation No. 471 from November, 23, 2011) are: average OEL = 0.03 mg m³ for 4,4⁻ -MDI, average OEL = 0.035 mg m³ for 1,6-HDI and average OEL = 0.014 mg m³ for 2,4and 2,6-TDI [19].

Monitoring of isocyanates exposure can be based on the air sampling, but also on analysis of isocyanate-derived diamines in urine or in hydrolyzed plasma [10, 20, 21]. Exposure to toluene diisocyanates and methylene diphenyl diisocyanate can be monitored in urine as toluene diamine and methylene dianiline, respectively [10].

Direct methods for the determination of DIs are correlated with the air sampling, since the sample collection, analytical processing and evaluation are complicated. The difficulties come from the determination of the very low concentrations of the highly reactive diisocyanate compounds, which are present in the forms of vapors and aerosols. Besides the main components it is necessary to monitor also other isomers, which are present in significant concentrations [1, 22].

The reliable determination of the diisocyanates in atmosphere needs appropriate air sampling and suitable sorbents. Sorption of DIs presents a problem in air sampling from a viewpoint of fixation reliability in absorption solution. Reliability of sampling techniques is demonstrated by validation of sampling method procedures. Validation is mostly based on mutual comparison of different methods, where laboratory and field tests might produce different results. The preparation of defined atmosphere similar to real situation in the workplace is difficult; testing of sampling methods in the model laboratory experiments is not reliable enough. Hence, existing methods for the determination of diisocyanantes, including 4,4⁻-MDI, in the workplace atmosphere can not be considered concurrently reliable and universal. So far development in this field can not be considered to be completed [1].

Several methods for the determination of diisocyanantes have been described in the literature. The most common methods are classic spectrophotometric methods, high performance liquid chromatography (HPLC) with UV, fluorometric, electrochemic or mass spectrometric detection, as well as gas chromatography with selectedion monitoring in the negative-ion chemical-ionization mode [23]. The usage of the spectrophotometric determination is limited due to problems in the sensitivity and selectivity [24]. Current methods include atmosphere sampling based on derivatisation of DIs to create stabile products, which can be determined by liquid chromatography. The method is recommended by specialized organizations: National Institute for Occupational Safety and Health (NIOSH) - methods No. 5521 (1994), 5522 (1998) and 5525 (2003). Analytical Methods of Occupational Safety and Health Administration (OSHA) - methods No. 42 (1983) and 47 (1984) in USA and Health and Safety Executive (HSE) methods - method MDHS 25/3 (1999) in Great Britain [21, 25-29]

The aim of this work was to verify the analytical HPLC method of the Occupational Safety and Health Administration (OSHA) for isocyanates determination at the laboratories of Authority of Public Health in Nitra, Slovak Republic (APH).

Esperimental part

Chemicals

Standards of diisocyanates - 2,4-toluene diisocyanate (95%), 2,6-toluene diisocyanate (97%), 1,6-diisocyanatohexane (98%), 4,4´-methylendiphenyl isocyanate (98%) were purchased from Supelco (Bellefonte, Pennsylvania, USA). Acetonitrile (ACN, 99.8% for HPLC) was purchased from Merck (Darmstadt, Germany), dimethyl sulfoxide (DMSO, 99.5% p.a.) was obtained from Fluka (Buchs, Switzerland) and ammonium acetate (99%, p.a.) was purchased from Centralchem (Bratislava, Slovakia).





Sampling and extraction procedure

Disocyanates were adsorbed by sampling on a glass fiber filter impregnated with 1-(2-pyridyl) piperazine (type SKC Cat. No. 225-9002) 37 mm filters with glass fibers coated with 1-(2-pyridyl)-piperazine placed in 3-piece styrene cassettes. The new filters were stored in a freezer to avoid decomposition of 1-(2-pyridyl) piperazine. Personal sampling pumps were hanged in a worker's breathing zone. Setting of low rate of air pumping allows calculation of air volume, passed through the pump. The sample pump drew the air through the 3-piece cassette. Aerosols were trapped on the PTFE filter while gases passed through to the next filter. Vapors were trapped on the fiber glass filter. A chemical reaction occured on this filter resulting in the formation of a highly detectable urea derivative. The sampling of the workplace atmosphere took usually 30 min, but the period can be shorter or longer depending on the DIs' concentrations expected. The filters were extracted immediately in acetonitrile (ACN)- dimethyl sulfoxide (DMSO) extraction solvent (ACN:DMSO = 90:10 v/v) and analyzed.

HPLC System

Concentrations of diisocyanates were determined by HPLC with UV detection. A Thermo Separation Products liquid chromatograph (Canton, Massachusetts, USA), with Thermo Separation Products P100 Pump for HPLC, a UV-150 UV-Vis detector, a column oven LCO 101 (Ecom, Prague, Czech Republic), and a 20µL sample loop. A CSW Workstation software was used to control the HPLC system and for data processing.

HPLC method

Chromatographic separations were performed on a Prodigy C8 column $(150 \times 4.6 \text{ mm}, 5 \mu\text{m})$, Phenomenex, USA). An UV detector operated at a wavelength of 254 nm under the isocratic conditions. For the separations of 2,4-TDI, 2,6-TDI, 1,6-HDI a mobile phase I (0.01 mol L⁻¹ CH₃COONH₄ in an ACN-water mixture (3:7, v/v) adjusted to *p*H 6.0-6.2 with acetic acid), and for determination of 4,4⁻-MDI a mobile phase II (0.01 mol L⁻¹ CH₃COONH₄ in ACN-water mixture (1:1, v/v) adjusted to *p*H 6.0- 6.2 with acetic acid) were used. The flow rate was 1.0 mL min⁻¹, the column temperature was 35°C. The injection volume was 20 µL for extracts and standard mixtures. Operating pressure was maximum 24 MPa, and total analysis time was 15 min. for 2,4-TDI, 2,6-TDI, 1,6-HDI and 7 min. for 4,4⁻-MDI.

Procedures

Standard solutions

From the dimethyl sulfoxide stock solutions of 2,4toluene diisocyanate, 2,6-toluene diisocyanate, 1,6diisocyanato-hexane, and 4,4´-methylene-diphenyl isocyanate (each 1.0 mg mL⁻¹), standard solutions were prepared in the concentration range from 0.4 to 2.0 mg mL⁻¹ by dilution of stock solution with acetonitrile mixtures. The solutions were stored at 4°C and used for a week.

Validation and verification of the method

Standard solutions were used for the construction of a calibration curve. All calculated validation characteristics were summarized in the Excel table and the final evaluation of the method. Calculation of the validation characteristics was performed according to a previously published work [30]. The analytical method was validated for the purpose of the laboratory of the Regional Authority of Public Health in Nitra, Slovak Republic.

Verification of the analytical method for sampling of workplace atmosphere in practice was performed in plants for car accessories production. Four workplaces with exposition of workers to DIs were monitored: workplace for metal components spraying with coating compositions, performed by spraying pistols (workplace I), pumping of technical DIs into smaller containers (workplace II), application of polyurethane glue by brush on a leather and caps of gear level and heating with warm air (workplace III), production of car accessories from hard foam (workplace IV).

Results and discussions

Coming from experiences in our region, diisocyanates present in the workplace atmosphere belong to two groups.

The first group includes the 2,4-TDI, 2,6-TDI and 1,6-HDI, and the second group 4,4⁻-MDI. These DIs are present together exceptionally. This fact has an important influence on the selection of the analytical method, the standards, the calibration, and the composition of the mobile phase or the elution mode (isocratic or gradient mode). The compounds in the first group can be eluted within a relatively short time applying mobile phase I, while 4,4⁻-MDI is retained stronger on a column sorbent. It is useful to change composition of the mobile phase or apply gradient elution to decrease the retention time of 4,4 -MDI. If DIs from the both groups is not present in the same sample, the time of analysis is shorter and amount of eluent used is lower, what results in cheaper analysis. Since the DIs of the two groups was not present always together in the samples, we used two different mobile phases for the analyses and applied them depending on the expected presence of the DIs from the two groups. According to the OSHA method No. 42 a mobile phase containing 0.01 mol dm^{-3} of CH₂COONH₄ in 3:7 (v/v) acetonitrile-water mixture is used for the determination of 2,4-TDI, 2,6-TDI and 1,6-HDI. The method no. 5522 NIOSH gives certain tolerance in composition of mobile phase, *i.e.* 0.01 mol dm⁻³ of CH₂COONH₄ in 40:60 to 50:50 (v/v) acetonitrile-water mixtures. Due to the composition of the real samples of atmosphere from our region for a satisfactory separation of the DIs we used a slightly different composition of the eluent, 0.01 mol dm⁻³ of CH₃COONH₄ in 3.7 acetonitrilewater (v/v). The determination of 4,4⁻-MDI was performed as described in the OSHA method no. 47. Separations of the diisocyanates are shown in figures 2 and $\overline{3}$.



Fig. 2. Chromatogram of 2,6-TDI (1), 1,6-HDI (2), 2,4-TDI (3)
(Mobile phase I: 0.01 mol L⁻¹ CH₃COONH₄ in an ACN-water mixture (3:7, v/v) adjusted to *p*H 6.0 – 6.2 with acetic acid, flow rate was 1.0 mL min⁻¹, injection volume was 20μL)



Fig. 3 Chromatogram of 4,4´-MDI (1). (Mobile phase II: 0.01 mol L⁻¹ CH₃COONH₄ in ACN-water mixture (1:1, v/v) adjusted to pH 6.0-6.2 with acetic acid, flow rate was 1.0 mL min⁻¹, injection volume was 20 μL.

DIa	Repeatability	LOD	LOQ	Recovery	urx				
DIS	%	μg m ⁻³	mg m ⁻³	%	%				
Determined for the mobile phase I.									
2,4-TDI	3.10	1.9	5.7	101	11				
1,6-HDI	2.98	1.1	3.4	103	12				
2,6-TDI	4.24	2.9	8.6	101	12				
Determined for the mobile phase II.									
4,4´-MDI	4.51	2.8	8.3	99	11				
Note:									

Table 1 SELECTED VALIDATION CHARACTERISTICS FOR DETERMINATION OF DIISOCYANATES

Table 2 SPRAYING OF METAL COMPONENTS BY COATING COMPOSITIONS WITH SPRAYING PISTOL (WORKPLACE I)

Table 3 PUMPING OF TECHNICAL DIS INTO SMALLER CONTAINERS (WORKPLACE II)

0

< 5.7

ND

ND

ND

8

< 5.7

ND

ND

ND

LOD - limit of detection calculated for 20 dm3 of atmosphere sampled, LOQ - limit of quantification calculated for 20 dm3 of atmosphere sampled, u_{rx} - relative standard uncertainty calculated for 20 dm³ of atmosphere sampled.

DIs	Content of diisocyanates µg m ⁻³					
	1	1 2 3		4		
2,4-TDI	8.6	11.2	9.8	9.3		
1,6-HDI	ND	ND	ND	ND		
2,6-TDI	< 8.5	< 8.5	ND	ND		
4,4´-MDI	ND	ND	ND	ND		
Note: ND value below limit of detection						

Note: ND – value below limit of detection

DIs	Content of diisocyanates µg m ⁻³					
	1	2	3	4		
2,4-TDI	8.8	10.5	11.2	8.5		
1,6-HDI	ND	ND	ND	ND		
2,6-TDI	8.6	< 8.5	9.1	ND		
4,4´-MDI	39.5	44.8	29.7	33.6		
Note: ND – value below limit of detection						

3

< 5.7

ND

< 8.5

ND

1	
į.	
-	Table 4
-	APPLICATION OF PU GUE BY

BRUSH AND HEATING WITH A WARM AIR (WORKPLACE III)

Note: ND - value below limit of detection									
DIs	Content of diisocyanates µg m ⁻³						Table 5		
	1	2	3	4	5	6	7	8	PRODUCTION OF CAR
2,4-TDI	ND	ND	ND	ND	ND	ND	ND	ND	ACCESSORIES FROM HARD
1,6-HDI	ND	ND	ND	ND	ND	ND	ND	ND	FOAMS – COMPARTMENTS OF
2,6-TDI	ND	ND	ND	ND	ND	ND	ND	ND	GEAR LEVELS (WORKPLACE IV)
4,4'-MDI	ND	8.3	9.7	11.1	< 8.3	10.1	8.8	< 8.3	

Content of diisocyanates μg m⁻³

5

5.1

ND

ND

ND

6

8.9

ND

< 8.5

ND

7

ND

ND

ND

ND

Note: ND - value below limit of detection

4

10.2

ND

8.7

ND

The reliability of the determinations was confirmed by validation for both groups of DIs with a use of validation characteristics listed in table 1. Model solutions of DIs standards were applied for the calculation of validation characteristics. The repeatability of the method (2.98 to 4.51%) is under the set limit of 5%. The relative standard uncertainty for the parameters was 11 - 12% with a recovery between 99 and 103%. The determination of the DIs was linear over the whole concentration range. Table 1 show that the LOD and LOQ values are low enough for a secure determination of diisocyanate concentrations, which are harmful for health in samples equal to 20 dm³ of atmosphere. The validation characteristics proved that the methods can be successfully applied for the determination of the above mentioned DIs. The validated analytical method was applied for the measurements of real samples. Results of analyses of workplace atmosphere obtained in

workplaces for production of car accessories are shown in tables 2 to 5. The samples collected from the breathing zone of 4 workers on the workplace I showed presence of 2,4-TDI and 2,6-TDI, in concentrations ranging from 8.6 to 11.2 μ g m⁻³. The concentrations of 1.6-HDI and 4.4⁻-MDI were mostly below the limit of detection of the methods used.

The workplace II included a pumping of technical DIs into smaller containers with the release of DIs to the atmosphere. The breathing zone of 4 workers contained the concentration of 2,4-TĎI ranging from 6.3 to 13.2 μ g m³, 2,6-TDI was present from 8.5 to 9.4 μ g m³ and 4,4 ²-MDI was found in a range from 29.7 to 44.8 μ g m⁻³.

The level of 1,6-HDI was found to be below the limit of detection, certainly due to the real composition of the mixtures pumped.

DIs

2,4-TDI

1,6-HDI

2,6-TDI

4,4´-MDI

1

ND

ND

ND

ND

2

< 5.7

ND

ND

ND

Very low concentrations of the diisocyanates were found in the samples obtained from 9 workers of workplace III, where the application of PU glue by brushing and heating of gear level with a warm air were done. All values were below the limit of detection or limit of quantitation.

From workplace IV were obtained the real samples of 8 workers, who worked in the production of car accessories made from hard foams (parts of gear level) showed concentrations for 4,4[']-MDI in the range from 8.3 to 13.3 μ g m³, with a mean value of 9.8 μ g m³. The concentrations of the other DIs (2,4-TDI, 2,6-TDI a 1,6-HDI) were below their limits of detection.

The highest diisocyanate concentrations were measured in workplace II, where a pumping of technical DIs into smaller containers was done. However, the short term concentration level of 4,4´-MDI raised up to the 149% of the OEL, but exposure was short term, therefore it cannot be considered as a level exceeding the limit of average exposure. Much lower concentrations were detected at the other technological processes. The results show good comparison to other results in this field [1, 31].

Conclusions

Methodology, to determine diisocyanataes was adjusted to the conditions of laboratories of APH using optimization and validation of HPLC measurements with a C8 column of 150 mm length. The mobile phase composition described in the international methods needed also optimization. The newly optimized method was validated using statistical analysis to evaluate the validation parameters, and verified on real atmosphere samples. This improved method is now applied routinely for the control of workplace atmosphere in plants with the production of car accessories in Slovakia.

This work presented the method of sampling and laboratory analysis of 2,4-toluene diisocyanate (2,4-TDI), 2,6-toluene diisocyanate (2,6-TDI), 4,4'-methylenediphenyl isocyanate (4,4'-MDI), and 1,6-hexamethylene diisocyanate (1,6-HDI). Air samples from working environment were collected in the breathing zone of exposed workers in plants with the production of car accessories.

Results in our work showed that the method, which was validated in the conditions of our laboratory, can be successfully used for the determination of DIs in workplace atmosphere. The LOD and LOQ values determined are low enough to determine the concentration of diisocyanates in the ranges where they can be harmful for health. The repeatability of the method, the calculated relative standard uncertainty, the recovery and the linearity of the calibration curve are as required for such measurements.

By testing the real conditions, we could conclude that the methods can be used for the analyses of real samples. Finally, we can conclude that the most diisocyanate levels did not exceed the occupational exposure limits in any of the monitored workplaces.

List of abbreviations

DIs- diisocyanates

- OEL occupational exposure limits for the gases, vapors and aerosols
- 1,6-HDI 1,6-hexamethylene diisocyanate

2,4-TDI - 2,4-toluene diisocyanate

2,6-TDI - 2,6-toluene diisocyanate

4,4´-MDI - 4,4-methylenediphenyl diisocyanate

DMSO -dimethyl sulfoxide

- ACN acetonitrile
- PU polyurethanes

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