Federation of the Employment Accidents Insurance Institutions of Germany (Hauptverband der Berufsgenossenschaften) Centre for Accident Prevention and Occupational Medicine Alte Heerstraße 111, 53757 Sankt Augustin Expert Committee Chemistry

Carcinogenic substances	Order number:	ZH 1/120.57E
Established methods	Issue:	June 1995

Method for the determination of 4,4'-methylene-bis(*N*,*N*-dimethylaniline)

Method tested and recommended by the Berufsgenossenschaften for the determination of 4,4'-methylene-bis(N,N-dimethylaniline) in working areas after discontinuous sampling. For the assessment of working areas, both personal or stationary sampling are possible:

Sampling with a pump and absorption on an impregnated filter, high performance liquid chromatography (HPLC) after desorption.
"4,4'-Methylene-bis(*N*,*N*-dimethylaniline)-1-HPLC" (Issue: June 1995)

IUPAC name:

4,4'-methylene-bis(*N*,*N*-dimethylaniline), bis-(4-dimethylaminophenyl)methane

CAS No: 101-61-1

1 Sampling with a pump and absorption on an impregnated filter, high performance liquid chromatography (HPLC) after desorption

This method permits the determination of 4,4'-methylene-bis(N,N-dimethylaniline) concentrations in working areas averaged over the sampling time after personal or stationary sampling.

Principle:	With a pump a measured air volume is drawn through a glass fibre filter impregnated with sulfuric acid. The adsorbed 4,4'-methylene- bis(<i>N</i> , <i>N</i> -dimethylaniline) is eluted with water and analysed by high performance liquid chromatography.			
Technical data:				
Quantification limit:	absolute: relative:	0.09 μ g 0.015 mg/m ³ for 500 L air sample, 4 mL desorption solution and 50 μ L injection volume.		
Selectivity:	The selectivity has to be checked in each individual case.			
Advantages:	Personal and selective measurements are possible.			
Disadvantages:	No indication of peak concentrations.			
Apparatus:	Pump equipped with gas meter or flow meter, acid-impregnated glass fibre filter with filter holder, HPLC apparatus equipped with UV detector.			

Detailed description of the method

Contents

- 1 Equipment, chemicals and solutions
- 1.1 Equipment
- 1.2 Chemicals
- 1.3 Solutions
- 1.4 Impregnation of the filters
- 2 Sampling
- 3 Analytical determination
- 3.1 Sample preparation and analysis
- 3.2 Operating conditions for high performance liquid chromatography
- 4 Evaluation
- 4.1 Calibration
- 4.2 Calculation of the analytical result
- 5 Reliability of the method
- 5.1 Accuracy
- 5.2 Quantification limit
- 5.3 Selectivity
- 6 Discussion
- 7 References

1 Equipment, chemicals and solutions

1.1 Equipment

Pump, flow meter or gas meter suitable for measuring flow rates of 3.5 L/min (e. g. Gilian PP5 ex, GSM GmbH, Neuss-Nerf)

Sampling head GSP (e.g. GSM GmbH, Neuss-Nerf or DEHA-Haan & Wittmer GmbH, Friolzheim)

Glass fibre filter (e. g. Schleicher & Schüll, No 6)

Gas meter, wet type (e. g. Ritter AG)

10, 100 and 1000 mL Volumetric flasks,

5 µL to 1 mL Adjustable volume pipettes (e. g. type Pipetman P, Abimed)

Sample vials

Polytetrafluoroethylene (PTFE) syringe prefilter (e. g. Millex FG 13 0.2 μ m, Millipore)

2.5 mL Disposable syringes

HPLC Apparatus equipped with gradient pump control and UV detector, recording and evaluation unit

Water purification unit (e.g. Elgastat, Elga, supplier in Germany: Labotec, Wiesbaden) Snap cap bottles

Ultrasonic bath

143

1.2 Chemicals

4,4'-Methylene-bis(*N*,*N*-dimethylaniline), 98%, (e.g. Aldrich) Water for HPLC (e.g. purified with the Elgastat (UHQ water)) Methanol (e. g. Lichrosolv, Merck, Darmstadt) Potassium dihydrogen phosphate, analytical grade Sulfuric acid, 0.5 mol/L, analytical grade

1.3 Solutions

Buffer solution:

Potassium dihydrogen phosphate solution, 30 mmol/L. 4.1 g potassium dihydrogen phosphate is dissolved in 1 L UHQ water.

Stock solution:

4,4'-Methylene-bis(N,N-dimethylaniline) solution of 2 mg/mL methanol. In a 50 mL volumetric flask 100 mg 4,4'-methylene-bis(N,N-dimethylaniline) is weighed to the nearest 0.1 mg and diluted to the mark with methanol.

Calibration solutions:

4,4'-Methylene-bis(N,N-dimethylaniline) solutions of 0.4, 2.0, 4.0, 12, 24 and 40 µg/mL water/methanol mixture.

2, 10, 20, 60, 120 and 200 μ L of the stock solution are each transferred to a 10 mL volumetric flask and diluted to the mark with a 1:1 mixture of UHQ water and methanol (v/v). With an air sample volume of 500 L a concentration range of 0.003 to 0.3 mg/m³ is covered by these solutions.

1.4 Impregnation of the filters

The glass fibre filters are immersed in sulfuric acid, predried by leaving in air for 30 minutes and completely dried at 40 °C in a drying cabinet. The impregnated filters are stored in a desiccator. They are stable for eight weeks.

2 Sampling

The sampling head GSP is equipped with an acid-impregnated glass fibre filter and connected to the pump. The pump and filter are carried by a person during working hours or used in a stationary position. The flow rate is adjusted to ensure the collection of the inhalable dust fraction in accordance to DIN EN 481 [1, 2]. With a flow rate of 3.5 L/min and a sampling time of 2.5 hours this corresponds to a sample volume of 525 L.

3 Analytical determination

3.1 Sample preparation and analysis

As sample preparation the filter is transferred to a snap cap bottle. 4 mL UHQ water is added. After 15 minutes ultrasonic treatment the solid parts are separated from the solution by filtering through a $0.2 \,\mu m$ PTFE syringe prefilter (desorption solution).

An unloaded impregnated filter is eluted with 4 mL water (blank solution) to ensure that the water used for desorption and the glass fibre filter are free from interfering impurities.

50 μ L is taken from the desorption solution, injected into the high performance liquid chromatograph and a chromatogram is recorded as described in Sect. 3.2. After chromatographic separation the 4,4'-methylene-bis(*N*,*N*-dimethylaniline) is detected at a wavelength of 260 nm.

3.2 Operating conditions for high performance liquid chromatography

Apparatus:	Hewlett Packard 1090 equipped with diode array detector		
	(DAD) and autosampler		
Column:	LiChroCART cartridge		
	Length:	250 mm	
	Internal diameter:	4 mm	
	Stationary phase:	LiChrospher 100 RP-18	
	Particle size:	5 μm	
Precolumn:	Length:	4 mm	
	Internal diameter:	4 mm	
	Stationary phase:	LiChrospher 100 RP-18	
	(e. g. from Merck, Darmstadt)		
Mobile phase:	Gradient elution		
	Starting with a mixture of 30% by volume of buffer solution		
	and 70 % by volume of methanol. The proportion of		
	methanol is increased continuously to 100% within 15 min.		
Flow rate:	0.5 mL/min		
Injection volume:	50 µL		
Detection wavelength:	260 nm		
Column oven temperature:	40 °C		

The method was characterized under the following experimental conditions.

4 Evaluation

4.1 Calibration

 $50 \ \mu$ L each of the calibration solutions (cf. Sect. 1.3) is injected into the chromatograph. The calibration curve is obtained by plotting the 4,4'-methylene-bis(dimethylaniline) concentrations of the calibration solutions against the peak areas measured. The linearity is checked. In the given concentration range the calibration curve is linear.

The calibration factor/is calculated according to equation (1) using the measured peak areas obtained for 4,4'-methylene-bis(dimethylaniline) with the various calibration solutions :

$$f = \frac{c_{\rm c}}{F} \tag{1}$$

Legend:

- f Calibration factor
- c_c 4,4'-Methylene-bis(*N*,*N*-dimetliylaniline) concentration in µg/mL in the calibration solution
- F Area of the 4,4'-methylene-bis(N,N-dimethylaniline) peak

The calibration factor is nearly the same for all dilutions. The mean value f must be used to calculate the analytical result.

4.2 Calculation of the analytical result

The concentration of 4,4'-methylene-bis(N,N-dimethylaniline) in the air sample in mg/m³ is calculated according to equation (2):

$$c_{\rm w} = \frac{V_{\rm D} \cdot F \cdot \bar{f}}{V \cdot \eta} \tag{2}$$

Legend:

- c_w 4,4'-Methylene-bis(*N*,*N*-dimethylaniline) concentration by weight in the air sample in mg/m³
- $V_{\rm D}$ Desorption volume: 4 mL
- F Area of the 4,4'-methylene-bis(N,N-dimethylaniline) peak for the desorption solution
- *f* Mean calibration factor for 4,4'-methylene-bis(*N*,*N*-dimethylaniline)
- V Air sample volume in L
- η Recovery rate

5 Reliability of the method

5.1 Accuracy

2.5, 25 and 50 μ L of the stock solution (cf. Sect. 1.3) are each transferred to an impregnated glass fibre filter and 500 L laboratory air is drawn through the filters as described in Sect. 2. For this air sample volume the amounts of 4,4'-methylene-bis(*N*,*N*-dimethylaniline) added to the filters correspond to air concentrations of 10, 100 and 200 μ g/m³. After 24 hours storage the filters were analysed as described in Sect. 3.1. Six determinations were carried out for each concentration. For the given concentrations the relative standard deviations were 3.9, 2.5 and 2.6%.

The recovery rate was 0.94 for an air sample volume of 500 L and a flow rate of 3.5 L/min.

5.2 Quantification limit

The absolute quantification limit is 0.09 μ g 4,4'-methylene-bis(*N*,*N*-dimethylaniline). The relative quantification limit is 0.015 mg/m for an air sample volume of 500 L, 4 mL of the desorption solution and 50 μ L injection volume.

5.3 Selectivity

The selectivity has to be checked in each individual case.

6 Discussion

At room temperature the shelf life of the loaded filters is at least three weeks.

In addition to 4,4'-methylene-bis(N,N-dimethylaniline), also airborne 4,4'-diaminodiphenylmethane (ZH 1/120.39 E), 3,3'-dimethyl-4,4'-diaminodiphenylmethane (ZH 1/120.51), o-toluidine (ZH 1/120.49) and aniline can be determined at workplaces under modified analytical conditions. The recovery rates are > 0.8.

7 References

- European Committee for Standardization (CEN) (1993) DIN EN 481, Workplace atmospheres

 Size fraction definitions for measurement of airborne particles. Brussels. Beuth Verlag, Berlin.
- [2] *Siekmann H, Blome H* (1994) Auswirkung der Europäischen Norm EN 481 auf die Probenahme von Partikeln in der Luft in Arbeitsbereichen. Staub-Reinhalt. Luft 54: 95–98.