Federation of the Employment Accidents Insurance Institutions of Germany
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Method for the determination of 1,5-diaminonaphthalene

Method tested and recommended by the Berufsgenossenschaften for the determination of 1,5-diaminonaphthalene in working areas after discontinuous sampling.
For the assessment of working areas, both personal and stationary sampling are possible:

1 Sampling with a pump and adsorption on an impregnated filter,
high performance liquid chromatography (HPLC) after desorption.
"1,5-diaminonaphthalene-1-HPLC"
(Issue: December 1998)

IUPAC name:	**CAS No:**
1,5-diaminonaphthalene, 1,5-naphthalenediamine	2243-62-1
Molecular weight:	**Molecular formula:**
158.20 g/mol	$C_{10}H_{10}N_2$

1 Sampling with a pump and adsorption on a filter, HPLC after desorption

This method permits the determination of 1,5-diaminonaphthalene 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 hydrochloric acid as stipulated in the definition of inhalable dust fraction [1]. The adsorbed 1,5-diaminonaphthalene is desorbed with a mixture of acetonitrile and aqueous ammonia and analysed by means of high performance liquid chromatography (HPLC).

Technical data:

Quantification limit: absolute: 20 ng 1,5-diaminonaphthalene,
relative: 0.016 mg/m^3 1,5-diaminonaphthalene for 500 L air sample, 5 mL desorbate (dilution in the ratio 1 : 1 v/v) and 25 µL injection volume.

Selectivity: Interfering components may cause too high values. In general, interferences can be eliminated by selecting different chromatography conditions.

Advantages: Both personal and selective sampling are possible.

Disadvantages: No indication of concentration peaks.

Apparatus: Pump,
gas meter or flow meter,
glass fibre filter impregnated with hydrochloric acid,
filter holder,
HPLC apparatus equipped with UV/VIS-detector.

Detailed description of the method

Contents

1 Equipment, chemicals and solutions

1.1 Equipment

For sampling:
Pump, suitable for a flow rate of 2 L/min (e.g. PP5 ex from Gilian, supplier in Germany: DEHA-Haan & Wittmer GmbH, 71288 Friolzheim)
Gas meter or flow meter
Filter holder (e.g. aerosol monitor, Catalogue No. M000 037 AO from Millipore, 65731 Eschborn)
Glass fibre filter, diameter 37 mm (e.g. No. 9 from Schleicher & Schüll, 37582 Dassel)

For sample preparation and analysis:
10, 25 and 50 mL Volumetric flasks
Adjustable pipettes, suitable for delivering 5 µL to 5 mL (e.g. Pipetman P from Abimed, 40736 Langenfeld)
20 mL Sample vials, amber
PTFE syringe prefilter (e.g. Millex FG13, 0.2 µm pore size from Millipore)
2 mL Disposable syringes

HPLC apparatus equipped with UV/VIS-detector
Data analysis device
Water-purification unit (e.g. Nanopure II from Barnsteadt, supplier in Germany: Wilhelm Werner GmbH, 51381 Leverkusen)
10 mL Glass vessels with snap-on caps, amber
Ultrasonic bath
Shaker (e.g. MTS 4 from IKA, 79219 Staufen)

1.2 Chemicals

1,5-Diaminonaphthalene, purity 97% (e.g. from Aldrich, 89552 Steinheim)
Aqueous ammonia, 25%, analytical grade (e.g. from Merck, 64271 Darmstadt)
Hydrochloric acid, 32%, analytical grade (e.g. from Merck, 64271 Darmstadt)

For HPLC:
Ultra pure water (e.g. prepared with the Nanopure II (UHQ water))
Acetonitrile, LiChrosolv (e.g. from Merck, 64271 Darmstadt)

1.3 Solutions

Desorption solution:
Mixture of 92 parts by volume of acetonitrile and 8 parts by volume of aqueous ammonia.

Solvent mixture 1:
Mixture of acetonitrile/water (1:1 v/v).

Solvent mixture 2:
Mixture of 96 parts by volume of solvent mixture 1 and 8 parts by volume of aqueous ammonia.

Stock solution:
Solution of about 1 mg of 1,5-diaminonaphthalene per millilitre solvent mixture 2.
About 25 mg of 1,5-diaminonaphthalene is weighed the nearest to 0.1 mg in a 25 mL volumetric flask. The flask is filled to the mark with the solvent mixture 2.

Calibration solutions:
Solutions of 0.5, 1.0, 5, 10, 15, 20 and 25 mg of 1,5-diaminonaphthalene per millilitre solvent mixture 1.
A few millilitres of solvent mixture 1 are added to each 10 mL volumetric flask. Then 5, 10, 50, 100, 150, 200 and 250 µL of the stock solution are each pipetted into one of the volumetric flasks and then the flasks are filled to the mark with solvent mixture 1. With these solutions and an air sample volume of 500 L a 1,5-diaminonaphthalene concentration range of 10 to 500 µg/m^3 is covered.
The stock solution and calibration solutions are not stable and must be freshly prepared before use.

1.4 Impregnation of the filter

The glass fibre filters are dipped into a mixture of 22 mL of UHQ water and 3 mL of 32% hydrochloric acid and dried in the air on a watch glass. The impregnated filters have a shelf life of at least four weeks.

2 Sampling

For sample collection a filter holder is equipped with two of the impregnated glass fibre filters and connected to the pump. The filter holder is wrapped in aluminium foil to exclude light. During working hours the pump and filter holder are carried by a person or used in a stationary position. The flow rate is set at 2 L/min in accordance with the definition of inhalable dust fraction [1]. With sampling for four hours this corresponds to an air sample volume of 480 L.

3 Analytical determination

3.1 Sample preparation and analysis

For sample preparation the filters are each placed in a glass vessel with a snap-on cap and 5 mL of desorption solution is added. After treatment in an ultrasonic bath and shaking (both for 15 minutes) solid parts are separated from the desorbate by filtering through a 0,2 µm PTFE syringe prefilter. An aliquot of these desorbates is then diluted with UHQ water in the ratio 1 : 1 v/v. This is imperative to avoid damaging the chromatography column.

To ensure that the water used for desorption and the glass fibre filter do not contain interfering substances, an unloaded impregnated filter is also prepared (blank solution).

25 µL is taken from each diluted desorbate, injected into the high performance liquid chromatograph and chromatogrammes are recorded as described in Sect. 3.2. After chromatographic separation 1,5-diaminonaphalene is detected at a wave length of 229 nm.

3.2 Operating conditions for high performance liquid chromatography

The method was characterized under the following experimental conditions:

Apparatus:	Hewlett Packard 1090 equipped with diode array detector (DAD) and autosampler.	
Pre-column:	Length:	30 mm
	Internal diameter:	4 mm

	Stationary phase:	250/8/4 Nucleosil 100-5 C_{18} AB from Macherey & Nagel
Column:	Length:	250 mm
	Internal diameter:	4 mm
	Stationary phase:	250/8/4 Nucleosil 100-5 C_{18} AB from Macherey & Nagel
Elution:	Isocratic	
Eluent:	Acetonitrile/UHQ water (20/80 v/v)	
Flow rate:	0.7 mL/min	
Injection volume:	25 µL	
Detection wavelength:	229 nm	
Column temperature:	40 °C	

4 Evaluation

4.1 Calibration

25 µL of each of the calibration solutions described in Sect. 1.3 are injected into the high performance liquid chromatograph and chromatogrammes are recorded. The calibration curve is obtained by plotting the measured peak areas against the 1,5-diaminonaphthalene concentrations contained in the various calibration solutions. The calibration curve is linear in the given concentration range.

4.2 Calculation of the analytical result

The 1,5-diaminonaphthalene concentration in the air sample in mg/m^3 is calculated according to equation (1):

$$c_\mathrm{w} = \frac{w}{V \cdot \eta} \tag{1}$$

c_w	Concentration by weight of 1,5-diaminonaphthalene in the air sample in mg/m^3
w	Sum of the 1,5-diaminonaphthalene weights in the desorbate in µg determined from the calibration curve
V	Air sample volume in L
η	Recovery rate

If more than 10% of the weight of 1,5-diaminonaphthalene deposited on the first filter is found on the second filter, sampling must be repeated with a smaller volume of air.

5 Reliability of the method

5.1 Accuracy and recovery

To determine the relative standard deviation of the procedure and the recovery,

- 20 µL of a solution of 0.66 mg of 1,5-diaminonaphthalene per millilitre solvent mixture 2,
- 20 µL of a solution of 2.64 mg of 1,5-diaminonaphthalene per millilitre solvent mixture 2,
- 20 µL of a solution of 5.07 mg of 1,5-diaminonaphthalene per millilitre solvent mixture 2

were each transferred to two impregnated glass fibre filters placed one behind the other. After the solutions were added the filter holders were wrapped in aluminium foil to exclude light. Then 500 L of laboratory air was drawn through the filters as described in Sect. 2. The spiked weights of 1,5-diaminonaphthalene correspond for the 500 l air volumes to concentrations of 0.026, 0.11 and 0.20 mg/m^3. The filters were then analysed as described in Sect. 3.1. Six individual determinations were carried out for each concentration. The relative standard deviations and recovery rates found are listed in the Table 1:

Table 1. Standard deviation (rel.) *s* and recovery rate.

Concentration mg/m^3	Standard deviation (rel.) *s* %	Recovery rate
0.026	3.9	0.92
0.11	1.9	0.94
0.20	1.8	0.96

5.2 Quantification limit

The absolute quantification limit is 20 ng of 1,5-diaminonaphthalene. It was determined from the signal/noise ratio of the blank value chromatogrammes.
The relative quantification limit is 0.016 mg/m^3 1,5-diaminonaphthalene for a 500 L air sample, 5 mL desorbate, dilution of the desorbate in a ratio of 1:1 v/v and 25 µL injection volume.

5.3 Selectivity

Interfering components may cause too high values. In general, interferences can be eliminated by selecting different chromatography conditions. In practice the separation conditions described above have proved reliable.

6 Discussion

The loaded filters can be stored in the dark at room temperature for at least a week without any loss of adsorbed 1,5-diaminonaphthalene.
In addition to 1,5-diaminonaphthalene also 1,2-phenylenediamine and 1,3-phenylenediamine can be determined in the workplace air with the method described.

7 References

[1] *European Committee for Standardization (CEN)* (1993) DIN EN 481, Workplace atmospheres – Size fraction definitions for measurement of airborne particles. Brussels. Beuth Verlag, Berlin.