HSE
Health and Safety Executive

Azodicarbonamide in air

Laboratory method with sample collection on to filters, solvent desorption and liquid chromatography

MDHS92/2

Methods for the Determination of Hazardous Substances

Health and Safety Laboratory

Scope

1 This procedure describes a method for the determination of time-weighted average airborne azodicarbonamide (CAS No. 123-77-3) concentrations in air. The air sample is collection onto a glass-fibre or polytetrafluoroethylene (PTFE) filter and analysed by high performance liquid chromatography (HPLC). It is suitable for both short-term (15 minute) and long-term (up to 8 hour) sampling durations.

Summary

2 A measured volume of air is drawn through a glass fibre or PTFE filter mounted in a respirable dust sampler. The sample is desorbed from the filter by sonication in a mixture of ethyl acetate and dimethyl sulphoxide. The resulting solution is analysed by HPLC with UV detection.

3 The use of alternative methods not included in the MDHS series is acceptable provided they can demonstrate the accuracy and reliability appropriate to the application.

Recommended sampling

4 For long-term exposures: maximum sampling time: 8 hours; sampling rate: 2 $l.min^{-1}$; sampled volume: up to 960 litres.

For short-term exposures: sampling time: 15 minutes; sampling rate: 2 $l.min^{-1}$; sampled volume: 30 litres.

Prerequisites

5 Users of this procedure will need to be familiar with the content of MDHS14.[1]

Safety

6 Users should be familiar with standard laboratory practice and carry out a suitable risk assessment. It is the user's responsibility to establish appropriate health and safety practices and to ensure compliance with regulatory requirements.

Equipment

7 Inhalable dust sampler as described in MDHS14.[1]

8 Personal sampling pumps that meet the requirements of BS EN 13137.[2]

9 Binder-free glass fibre or PTFE filters of appropriate diameter for use in the selected inhalable dust sampler.

10 A portable flow meter, calibrated against a primary standard, with a measurement uncertainty typically less than ±2%.

11 Flexible plastic tubing for making a leak-proof connection from the sampling head to the pump; belts or harnesses to facilitate attachment of sampling apparatus to sample subjects; flat-tipped tweezers for loading and unloading the filters into samplers; and filter transport cassettes to transport samples to the laboratory.

Laboratory apparatus and reagents

12 During the analysis use only reagents of a recognised analytical grade.

13 Ethyl acetate: HPLC grade.

14 Dimethyl sulphoxide (DMSO): HPLC grade.

15 Azodicarbonamide.

16 Laboratory detergent solution: A laboratory grade detergent suitable for cleaning of samplers (if suitable) and lab-ware, diluted with water according to the manufacturer's instructions.

17 A selection of laboratory glassware, including Pasteur pipettes; beakers; cleaned with laboratory detergent by soaking overnight followed by rinsing with distilled water; 4 ml HPLC vials (with screw caps and Teflon coated septa), and volumetric flasks, Class A, complying with the requirements of BS EN ISO 1042.[3]

18 Positive displacement micropipettes complying with the requirements of BS EN 8655-6.[4]

19 Solvent-resistant plastic syringe filters (2 µm pore size) for sample filtration prior to analysis if required.

20 A balance, calibrated against a primary standard, for the preparation of the calibration standards. The balance should be capable of weighing to ±0.1 mg over the range 0 to 100 g.

21 HPLC system with UV or diode array detector. Suitable operating conditions are listed below but the use of other columns and conditions are acceptable provided they have the accuracy and reliability appropriate to the application:

Column dimensions	25 cm x 4.5 mm ID
Column packing	Silica, 5 µm
Mobile phase	95% ethyl acetate; 5% DMSO
Flow rate	1.5 $ml.min^{-1}$
UV detector	270 nm (major); 425 nm (minor)
Injection volume	20 µl

22 Under the above conditions, the retention time of azodicarbonamide was approximately 5 minutes.

Preparation and sampling

23 Sampling should be carried out in accordance with the procedures described in MDHS14[1] for inhalable dusts. Maximising the air volume will improve the detection limit for the procedure. Select a sampling period that avoids overloading or use two or more consecutive samples.

24 After sampling remove the filter from the sampling head using clean flat-tipped tweezers, place in a labelled transport container for transfer to the laboratory.

25 A minimum of three field blanks should be included with a batch of samples.

Calibration

26 Prepare stock solutions of azodicarbonamide as follows: into a 10 ml volumetric flask, weigh out accurately around 50 mg of azodicarbonamide and make up to volume with DMSO (5 $mg.ml^{-3}$ solution). Pipette 1 ml of the 5 $mg.m^{-3}$ solution into a second 10 ml volumetric flask and make up to volume with DMSO (500 $\mu g.ml^{-1}$ solution). Pipette 1 ml of the 500 $\mu g.ml^{-1}$ solution into a third 10 ml volumetric flask and make up to volume with DMSO (50 $\mu g.ml^{-1}$ solution). These solutions should be freshly prepared immediately prior to analysis.

27 Prepare at least six calibration standards to cover the range 0–1000 µg/ml azodicarbonamide (0–100 $\mu g.ml^{-1}$ for 15-minute samples) by pipetting appropriate volumes (up to 0.75 ml) of the azodicarbonamide stock solutions into labelled 4 ml HPLC vials, then adding sufficient DMSO to make the volume up to 0.75 ml, and finally 2.25 ml of ethyl acetate. A minimum of two calibration standards should be prepared from each stock solution.

28 Analyse the calibration solutions by HPLC in an identical manner to the samples and measure the peak areas of the target compound at 270 nm and 425 nm. Plot the peak areas against the corresponding azodicarbonamide concentration of the standard, in $\mu g.ml^{-1}$, and construct the lines of best fit. The slopes of these lines are the detector response factors (R_F) for azodicarbonamide at 270 nm and 425 nm (the R_F at 270 nm is typically around 20 times greater than that at 425 nm).

29 Modern HPLC equipment is usually sufficiently stable that a new calibration is not required with each set of samples. However, to verify the equipment, a quality assurance (QA) solution of known concentration, must be analysed with each set of samples. The QA solution may be prepared using the procedure above (paragraph 27).

Analysis

30 Analyse samples and blanks in an identical manner.

31 Transfer the sample or blank filter to a labelled 4 ml HPLC vial using clean flat-tipped tweezers.

32 Add 0.75 ml of DMSO and 2.25 ml of ethyl acetate, cap and place in an ultrasonic bath for 60 minutes. If the sample appears cloudy after ultra sonic

treatment (due to the presence of inorganic or other insoluble material), filter using a syringe filter into a second labelled 4 ml HPLC vial and re-cap.

33 Analyse the sample and blanks solutions by HPLC in an identical manner to the standards.

34 Measure the peak area of the target compound at 270 nm and 425 nm and convert these peak areas to an azodicarbonamide concentration, in $\mu g.ml^{-1}$, by dividing by the appropriate RF value obtained from the calibration standards. Where blank corrected concentrations at 425 nm are above the detection limit, calculate the mean azodicarbonamide content using peak areas at both analytical wavelengths; otherwise use only the value obtained at 270 nm.

35 Where high azodicarbonamide concentrations are found, dilute the sample solutions with 75:25 ethyl acetate-DMSO to bring the concentration back within the calibration range. Record the dilution factor and repeat the analysis.

Calculation of results

Calculate the volume, V_S, in m^3, of each air sample by multiplying the mean volumetric flow rate in cubic metres during the sampling period, by averaging the flow rate measurements taken at the start and end of the sampling period, by the sampling time in minutes.

Calculate the azodicarbonamide concentration in each air sample, C, in $mg.m^{-3}$, using the equation:

$$C = (D \times M_S) / V_S$$

where:

M_S = Concentration of azodicarbonamide in the sample solution, in $\mu g.ml^{-1}$

D = Desorption volume of the sample filter, in ml (3 ml)

Appendix: Additional information

Detection limit of the method

1 The qualitative and quantitative detection limits for azodicarbonamide, defined as three times and ten times the standard deviation of the blank determination, are typically around 1 and 3 µg per sample respectively. For a 15-minute air sample collected at 2 $l.min^{-1}$, these figures correspond to qualitative and quantitative detection limits of around 35 $\mu g.m^{-3}$ and 100 $\mu g.m^{-3}$ respectively, while for an 8-hour sample collected at the same flow rate the equivalent figures are 1 $\mu g.m^{-3}$ and 3 $\mu g.m^{-3}$ respectively.

Overall uncertainty

2 The overall uncertainty for this measurement procedure as defined in BS EN 482[5] is given below. These values were calculated from six replicate analyses at each level and include 5% for the uncertainty in pump flow rate measurement.

Sampling period	Measurement range (mg. m^{-3})	Overall uncertainty (%)	Performance requirements (%)[5]
15 minutes	0.1 – 0.5	20 – 30	-
	0.5 – 2.0	15 – 20	<30%
8 hours	0.1 – 0.5	15 – 20	<50%
	0.5 – 2.0	10 – 15	<30%

Interferences

3 Any compound that elutes in the region of the chromatogram containing the azodicarbonamide peak is a potential interferent. To reduce the likelihood of false positive readings the sample should be analysed at the two recommended wavelengths, 270 nm (major peak) and 425 nm (minor peak). The 270 nm peak is used as the main analytical wavelength while the 425 nm peak is used as a confirmation wavelength. No co-eluting components have been identified during laboratory tests or field trials, although where doubts over chromatographic peak identities persist, the use of a diode array detector may be required.

Stability

4 Storage experiments in the laboratory have shown that when sealed in containers, samples on glass-fibre or PTFE filters were stable for at least 1 month. Once desorbed, however, these should be analysed within 3–4 days as azodicarbonamide solutions left to stand for one week show deterioration.

References

1 *General methods for sampling and gravimetric analysis of respirable, thoracic and inhalable aerosols* MDHS14/4 HSE 2014 www.hse.gov.uk/pubns/mdhs/index.htm

2 BS EN 13137:2013 *Workplace atmospheres. Pumps for personal sampling of chemical agents Requirements and test methods* British Standards Institution

3 BS EN ISO 1042:2000 *Laboratory glassware: One-mark volumetric flasks* British Standards Institution

4 BS EN ISO 8655-6:2002 *Piston-operated volumetric apparatus. Gravimetric methods for the determination of measurement error* British Standards Institution

5 BS EN 482:2012 *Workplace exposure. General requirements for the performance of procedures for the measurement of chemical agents* British Standards Institution

You should use the current edition of any standards listed.

Further information

For information about health and safety, or to report inconsistencies or inaccuracies in this guidance, visit www.hse.gov.uk/. You can view HSE guidance online and order priced publications from the website. HSE priced publications are also available from bookshops.

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