

MDHS

*Methods for the Determination of
Hazardous Substances*
Health and Safety Laboratory



93

Glutaraldehyde in air

Laboratory method using high performance
liquid chromatography

October 1998

INTRODUCTION

Requirements of the COSHH Regulations

1 The Control of Substances Hazardous to Health (COSHH) Regulations¹ require that the health risk created by work involving hazardous substances should be assessed and exposure to such substances then prevented or controlled. The COSHH Regulations also include a requirement to provide suitable and sufficient information, instruction and training. Employers must therefore ensure that the requirements of the COSHH Regulations are fully satisfied before allowing employees to undertake any procedure described in this method.

Properties and uses

2 Glutaraldehyde is a colourless oil with a characteristic odour. It boils at about 188°C. Pure glutaraldehyde is seldom used as such but its solutions are widely used as sterilising agents for medical equipment that cannot be autoclaved. The other main use is as a tanning agent for leather.

Health effects

3 Glutaraldehyde is an irritant and a respiratory sensitiser which may cause asthma. Classification under the Chemicals (Hazard Information and Packaging for Supply) Regulations 1994 includes the risk phrase R42, may cause sensitisation by inhalation.

Exposure

4 The predominant occupational exposure to glutaraldehyde occurs in the health industries where it is used as a sterilising agent.

Analytical methods

5 This is not a 'reference' method in the strict analytical sense of the word. There are frequently several

alternative methods available for the determination of a particular analyte. With the exception of a few cases, where an exposure is linked to a specific method (eg rubber fume or asbestos), the use of methods not included in the MDHS series is acceptable provided that they have been shown to have the accuracy and reliability appropriate to the application.

6 This method has been validated to demonstrate that it is capable of meeting the stated performance parameters. If an alternative method is used it is necessary to determine and state performance parameters for that method.

SCOPE

7 This MDHS describes a method for the determination of glutaraldehyde in workplace air. It is suitable for concentrations from 0.01 ppm to 0.4 ppm and for sample periods of 15 minutes to eight hours.

Note 1: HSE Guidance Note EH42² advises employers about how they should conduct investigations into the nature, extent and control of exposure to substances hazardous to health which are present in workplace air. The objective of air monitoring is usually to determine worker exposure, and therefore the procedures described in this method are for personal sampling in the breathing zone. The method may also however be used for background or fixed location sampling.

Detection limits

8 The qualitative and quantitative detection limits for glutaraldehyde, defined as three times and five times the standard deviation of a blank determination, are typically around 0.3 µg and 1 µg per sample respectively. For a 3 litre air sample, these figures correspond to qualitative and quantitative detection limits of 0.1 µg m⁻³ and 0.3 µg m⁻³ respectively.

Overall uncertainty

9 The overall uncertainty for the method is determined using the following CEN definition³ for each batch of samples:

$$\text{Overall Uncertainty} = (2 \times \text{Precision}) + |\text{Bias}|$$

10 The bias on this method is essentially zero and thus the overall uncertainty of the method is due to the sampling errors and the analytical errors. The analytical precision depends on the amount of analyte and is of the order of 5% at levels corresponding to the OES. The sampling error is typically 10% and thus the overall uncertainty is about 11%.

Interferences

11 Heptanone and camphor form DNPH derivatives that coelute with the glutaraldehyde derivative and will thus interfere with the determination. Large concentrations of other materials that react with DNPH will also interfere.

Stability

12 The samples have been found to be stable for a month.

PRINCIPLE

13 A measured volume of air is drawn through a filter coated with a solution of DNPH in phosphoric acid. The glutaraldehyde reacts and gives a derivative that is desorbed from the filter with acetonitrile and separated from the excess reagent by HPLC. The derivative is quantified by monitoring the UV absorbance.

REAGENTS

14 During the analysis, use only reagents of a recognised analytical grade. Suitable personal protection (eg gloves and safety spectacles) should be used when handling the reagents listed below.

Water

15 Water complying with the requirements of BS 3978 grade 1 water.

Acetonitrile

16 HPLC grade acetonitrile.

2,4 Dinitrophenyl hydrazine

17 Dinitrophenyl hydrazine (DNPH) purified by double recrystallisation, as the hydrochloride, from about 5M hydrochloric acid, conversion back to the free base with aqueous ammonia, recrystallisation from acetonitrile rinsing with ether and drying.

Ammonia

18 Analytical reagent grade ammonia solution 25% w/w.

Hydrochloric acid (about 5M)

19 Dilute 80 ml of analytical reagent grade 37% w/w HCl to 200 ml with water.

Phosphoric acid

20 HPLC grade 85% phosphoric acid.

Ether

21 Analytical reagent grade diethyl ether.

Ethanol

22 Analytical reagent grade ethanol.

Glutaraldehyde

23 50% glutaraldehyde solution in water.

Laboratory detergent solution

24 A laboratory grade detergent suitable for cleaning of samplers and labware, diluted with water according to the manufacturer's instructions.

Gases

25 Helium 99.996%.

SAMPLING EQUIPMENT

26 Sampling and chromatographic equipment is available from the usual suppliers.

Sampling heads

27 37 mm diameter filter holders.

Filters

29 37 mm diameter glass fibre filters coated with dinitrophenyl hydrazine as per Appendix 1.

Sampling pumps

30 Sampling pumps, with an adjustable flow rate, incorporating a flow-meter or flow fault indicator, capable of maintaining the appropriate flow rate (200 ml/min) to within 5% of the nominal value throughout the sampling period (paragraph 37), and capable of being worn by persons without impeding normal work activity.⁴ The pumps shall give a pulsation-free flow (if necessary, a pulsation damper shall be incorporated between the sampling head and the pump, as near to the pump as possible). Flow-stabilised pumps may be required to maintain the flow rate within the specified limits.

Flow-meter

31 Flow-meter, portable, capable of measuring the appropriate flow rate (200 ml/min) to within $\pm 5\%$, and calibrated against a primary standard.⁴

Note 2: *The flow-meter incorporated into the pump may be used provided that it has adequate sensitivity, that it has been calibrated against a primary standard with a loaded sampler in line, and that it is read in a vertical orientation if it is of the supported float type. However, it is important to ensure that there are no leaks in the sampling train between the sampling head and the flow-meter, since in this event a flow-meter in the pump or elsewhere in line will give an erroneous flow rate.*

Note 3: *A soap bubble flow-meter may be used as a primary standard, provided its accuracy is traceable to national standards (see Appendix 2).*

Ancillary equipment

32 Flexible plastic tubing of such diameter as to provide leak-free connection from the pump to the sample holder; belts or other means by which the sampling pump can be fixed. Flat tipped tweezers for handling filters.

LABORATORY APPARATUS

Glassware

33 A selection of laboratory glassware, including beakers, measuring cylinders and class A volumetric flasks, complying with the requirements of BS 1792.⁵

Disposable gloves

34 Disposable gloves, to reduce the likelihood of contamination from the hands and to protect them from contact with harmful substances. Nitrile rubber gloves are suitable but it should be noted that any disposable gloves give limited protection.

Balance

35 A balance, calibrated against a traceable standard, for the preparation of the calibration standards. The balance should be capable of weighing to ± 0.001 mg over the range 0 to 40 mg

Micropipettes

36 A set of adjustable positive displacement micropipettes, calibrated against a primary standard, for the preparation of calibration and sample solutions.⁶

SAMPLING

Sampling procedure

37 For long-term samples, select a sampling period of an appropriate duration, such that the filter does not

become overloaded with particulate material (note that an 8-hour time weighted average concentration may be derived from the results of two or more consecutive samples, as described in Guidance Note EH42²).

Preparation of sampling equipment

38 Clean the sampling heads before use. Disassemble the samplers, soak in laboratory detergent solution (paragraph 24), rinse thoroughly with water, wipe with absorptive tissue and allow to dry thoroughly before reassembly.

39 Load the filters (coated as in Appendix 1) into clean, dry sampling heads (paragraph 27) using clean flat-tipped tweezers (paragraph 32). Connect each loaded sampling head to a sampling pump (paragraph 30) using plastic tubing (paragraph 32), ensuring that no leaks can occur. Switch on the pump, attach the calibrated flow-meter (paragraph 31) to the sampling head so that it measures the flow through the sampler inlet orifice, and set the appropriate flow rate (200 ml/min) with an accuracy of $\pm 5\%$. Switch off the pump and seal the sampler to prevent contamination during transport to the sampling position.

Collection of samples

40 Fix the sampling head to the worker, on the lapel and as close to the mouth and nose as possible.⁴ Then, either place the sampling pump in a convenient pocket or attach it to the worker in a manner that causes the minimum inconvenience, eg to a belt around the waist (paragraph 30). When ready to begin sampling, unseal the sampling head and switch on the pump. Record the time at the start of the sampling period, and if the pump is equipped with an elapsed time indicator, ensure that this is set to zero.

41 Since it is possible for a filter to become clogged, monitor the performance of the sample periodically, a minimum of every two hours (or more frequently if heavy filter loadings are suspected). Measure the flow rate with the calibrated flow-meter and record the measured value. Terminate sampling and consider the sample to be invalid if the flow rate is not maintained to within $\pm 5\%$ of the nominal value throughout the sampling period.⁴

Note 5: *Regular observation of the flow fault indicator is an acceptable means of ensuring that the flow rate of flow-stabilised pumps is maintained satisfactorily, provided that the flow fault indicator indicates malfunction when the flow rate is outside $\pm 5\%$ of the nominal value.*

42 At the end of the sampling period, measure the flow rate with an accuracy of $\pm 5\%$ using the calibrated flow-meter (paragraph 31), switch off the sampling pump, and record the flow time and the time. Also observe the reading on the elapsed time indicator, where fitted, and consider the sample to be invalid if the reading on the elapsed time indicator and the timed interval between switching on and switching off the sampling pump do not agree to within $\pm 5\%$, since this may suggest that the sampling pump has not been operating throughout the

sampling period. Disconnect the sampler from the sampling pump and reseal it.

43 Carefully record the sample identity and all relevant sampling data (see Appendix 4). Calculate the mean flow rate by averaging the flow rate measurements throughout the sampling period and calculate the volume of air sampled, in litres, by multiplying the flow rate in litres per minute by the sampling time in minutes.

44 With each batch of samples, submit for analysis at least three (coated but unused) filters from the same lot of filters used for sample collection. Subject these blank filters to the same handling procedure as the samples, but draw no air through them.

Transportation

45 Send the samplers for analysis in a container which will protect the samples in transit and which is labelled to ensure proper handling

ANALYSIS

46 Wear disposable gloves (paragraph 34) during analysis to reduce the possibility of contamination and to protect the hands from harmful solvents/reagents.

Cleaning of glassware

47 Before use, clean all glassware (paragraph 33) to remove any residual grease or chemicals. Firstly soak overnight in laboratory detergent solution (paragraph 24) and then rinse thoroughly with water and dry.

Preparation of calibration standards

48 Prepare at least five calibration standards to cover the range 0.2 to 40 µg of glutaraldehyde per ml as the DNPH derivative as follows. Weigh out aliquots of the glutaraldehyde derivative prepared in Appendix 4 and quantitatively transfer them to 50 ml volumetric flask and make up to volume with acetonitrile. Suitable masses of the derivative are approximately 40 µg, 100 µg, 500 µg, 2 mg, 10 mg. Alternatively the two most dilute standards can be prepared by dilution.

Chromatography

49 The chromatographic conditions used are;

Column: Zorbax CN 5µ, 4.6 mm, 25 cm
Mobile phase: 45:55 Buffer : acetonitrile
(The buffer being 1% w/v NaH₂PO₄•2H₂O in water adjusted to pH 5.5)
Flow rate: 2 m/min
Detector: UV at 360 nm
Injection volume: 10 µl

System calibration

50 The system is calibrated by injecting 10 µl aliquots of known concentrations of the glutaraldehyde derivative.

Samples

51 Sample filters are removed from their holders using tweezers and placed in 4 ml vials. 2 ml of acetonitrile are added and the vials capped with septum sealed caps. The samples are then left for an hour to desorb.

CALCULATION OF RESULTS

Volume of air sample

52 Calculate the volume, V_s, in litres, of each air sample (paragraph 43).

Concentration of glutaraldehyde in air

53 Calculate the glutaraldehyde concentration in each air sample, ρ(glutaraldehyde) in milligrams per cubic metre (mg m⁻³), using the equation:

$$\rho(\text{glutaraldehyde}) = \frac{(m - m_{\text{blank}}) \times 1000}{V_s \times 4.60}$$

Where m is the mass (µg) of glutaraldehyde derivative found on the filter and m_{blank} is the mean mass (µg) found on a blank filter.

TEST REPORT

54 Appendix 4 gives recommendations for information to be included in the test report.

APPENDIX 1

Coating filters

Note that acetonitrile is toxic and volatile and thus should be used in a fume cupboard.

Prepare a solution of 10 ml of 85% phosphoric acid in 90 ml of acetonitrile.

Prepare a coating solution by adding 25 mg of DNPH to a 25 ml flask, add 5 ml of the diluted phosphoric acid and make up to volume.

Support a sufficient number of filters on the rims of 25 ml glass beakers and pipette 0.5 ml of the coating solution onto each of them. Leave the filters to dry then transfer them to an airtight container for storage.

APPENDIX 2

Primary standard for calibration of portable flow-meter

The primary standard should preferably be a flow-meter whose accuracy is traceable to national standards, used with careful attention to the conditions of the calibration certificate. A bubble flow-meter may also be used. This is

an arrangement whereby the pump under test draws a soap film up a calibrated tube. The passage of the film is accurately timed between two marks whose separation defines a known volume. The volume between the marks can be checked by filling the burette with water, allowing temperatures to stabilise, drawing off a known volume and weighing the water, making allowance for the dependence of volume on temperature. A suitable bubble solution can be made by mixing one part of concentrated washing-up liquid, two parts glycerol and four parts water. The burette must be thoroughly wetted with the solution and several attempts at drawing the film up the tube may be necessary before the tube is wet enough for this to be achieved consistently. Traceability of the calibration will require checking of the clocks and use of certificated weights.

APPENDIX 3

The glutaraldehyde derivative is prepared as follows. Convert 6 g of DNPH to its hydrochloride by recrystallising from 100 ml of hot 5 M HCl. Wash the crystalline product with acetonitrile and then with ether. Leave it to dry. Dissolve 2 g of this product in 10 ml of HCl and 180 ml of hot ethanol. Mix 1 ml of 50% glutaraldehyde with 10 ml of ethanol and add this to the DNPH solution. Leave the mixture to cool and filter off the product. Recrystallise the product from boiling acetonitrile. Wash the crystals with ether and dry in air. Place the product in a sealed bottle and label it with the date of preparation. The product is stable for a year stored under ambient conditions

APPENDIX 4

Recommendations for the test report

It is recommended that the test report should include the following information:

- (a) a complete identification of the air sample, including the date of sampling, the place of sampling, and the identity of the individual whose breathing zone was sampled;
- (b) a reference to this MDHS and a description of any deviation from the procedures described;
- (c) the type and diameter of filter used, and the type of sampling head;
- (d) the type of sampling pump and flow-meter used, the primary standard against which it was calibrated, and the range of flow rates for which the flow-meter was calibrated;
- (e) the duration of the sampling period in minutes and/or the time at the start and at the end of the sampling period;
- (f) the volume of air sampled, in litres;
- (g) the name of the person who collected the sample;
- (h) the time-weighted average (TWA) concentration found in the air sample, in milligrams per cubic metre and/or the total mass collected on the filter, in milligrams;
- (i) the overall uncertainty of the method;
- (j) the name of the analyst;
- (k) the date of the analysis.

ADVICE

Advice on this method and the equipment used can be obtained from the Health and Safety Executive, Health and Safety Laboratory, Broad Lane, Sheffield S3 7HQ (telephone 0114 2892000).

The Health and Safety Executive wishes, wherever possible, to improve the methods described in this series. Any comments that might lead to improvements would therefore be welcome and should be sent to the above address.

REFERENCES

- 1 Health and Safety Commission *Control of Substances Hazardous to Health/Control of Carcinogenic Substances Approved Codes of Practice* HSE Books 1997 ISBN 0 7176 1308 9
- 2 Health and Safety Executive Environmental Hygiene Series Guidance Note *Monitoring strategies for toxic substances* EH42 HSE Books 1989 ISBN 0 11 885412 7
- 3 Comité Européen de Normalisation *Workplace atmospheres: general requirements for the performance of procedures for the measurement of chemical agents* BS EN 482
- 4 Health and Safety Executive *General methods for the gravimetric determination of respirable and total inhalable dust* MDHS 14/2 HSE Books 1997 ISBN 0 7176 1295 3
- 5 British Standards Institution *Specification for one-mark volumetric flasks* BS 1792 BSI 1993 ISBN 0 580 127540
- 6 British Standards Institution *Piston and/or plunger operated volumetric apparatus (POVA) Part 3: Methods of test* BS 7653-3 BSI 1993 ISBN 0 580 221 253

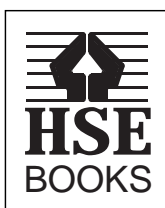
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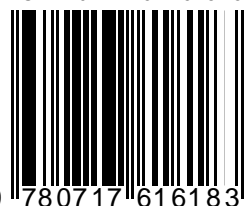
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