HSE
Health and Safety Executive

# MDHS 102

*Methods for the Determination of Hazardous Substances*

Health and Safety Laboratory

# Aldehydes in air

## Laboratory method using high performance liquid chromatography

May 2010

## Scope

1 This procedure – based on BS ISO 16000-3:2001[1] – describes a laboratory method for the determination of airborne concentrations of aldehydes using high performance liquid chromatography (HPLC). The method is only suitable for aldehydes that both react to give a stable reaction product with 2,4-dinitrophenylhydrazine (DNPH) during air sampling and which are amenable to the stated analytical conditions. The method is primarily intended for measurement of formaldehyde but is also suitable for the measurement of the other aldehydes listed in BS ISO 16000-3* – acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde, isovaleraldehyde, hexanal, crotonaldehyde, benzaldehyde, 2,5-dimethylbenzaldehyde and o-, m-, p-tolualdehyde and glutaraldehyde.

2 The method is typically applicable to both 15-minute and 8-hour sampling for airborne concentrations in the range 0.01 mg/m$^3$ – 10 mg/m$^3$.

## Summary

3 The aldehyde present in a measured volume of air is collected onto a DNPH-coated glass fibre filter or sorbent tube. These are usually pumped samples, however commercial diffusive samplers are also available. After sampling, the samplers are solvent desorbed into acetonitrile and the aldehyde derivatives analysed using HPLC with a photodiode array detector (PDA). Separation is achieved using a C18 column (3.9 × 300 mm) maintained at a temperature of 50 °C.

## Recommended sampling

4 For pumped samples on filters with expected exposures up to 2 mg/m$^3$: max sampling time: 8 hours; sampling rate: 0.1 to 1.0 l/min; sampled volume: 1.5 to 480 l.

5 For commercial samplers, in accordance with the manufacturers recommendation.

## Prerequisites

6 Users of this method will need to be familiar with the content of MDHS14[2].

## Safety

7 People using this method should be familiar with normal laboratory practice and carry out a suitable risk assessment. The method does not address all safety problems associated with its use. It is the user's responsibility to establish appropriate health and safety practices and to ensure compliance with regulatory requirements.

## Equipment

8 Sampling medium: DNPH-coated glass fibre filters contained in either a suitable personal inhalable dust sampler[2] or commercial diffusive sampler; DNPH-coated silica gel contained in a commercial sampling tube.

9 Sampling pumps: Capable of sampling up to 1 l/min and conforming to the requirements specified in MDHS14[2]. (Note: Some pumps may be unable to draw the higher flow rate through some samplers).

10 Belts, harnesses and tubing: To support sampling pumps and connect them with the sampling media.

11 Flow meter: Calibrated against a primary standard, ie a flow meter with accuracy traceable to national standards and conforming to the requirements of MDHS14[2].

12 Glassware: A selection of laboratory glassware including beakers and volumetric flasks.

13 Analytical balance: Capable of weighing to ± 0.1 mg over the range 0–100 g and calibrated against a traceable standard.

14 Pipettes: A range of adjustable positive displacement micropipettes to measure volumes in the range 200 µl to 2.0 ml. These should be tested in accordance with BS EN ISO 8655[3] or equivalent.

15 HPLC vials: 4 ml capacity; glass screw top.

16 Septa: Teflon-silicone septa for the HPLC vials.

17 HPLC system: Equipped with a photodiode array detector.

## Reagents

18 Water: Complying with the requirements of BS 3696 grade 1[4].

19 Acetonitrile: HPLC grade.

20 2,4-Dinitrophenylhydrazine (DNPH): purified using the procedure in Appendix 1.

21 Ammonia solution: Analytical reagent grade, 25% w/w.

22 Hydrochloric acid: Analytical reagent grade, 37% w/w.

23 Phosphoric acid: HPLC grade, 85% w/w.

24 Diethyl ether: Analytical reagent grade.

25 Ethanol: Analytical reagent grade.

26 Sodium dihydrogen phosphate: Analytical reagent grade.

27 Either the aldehydes themselves or their DNPH derivatives.

## Sample collection

28 If pumped filters are used for sampling, load the filter into the sampler and accurately set the flow rate to an appropriate flow rate between 0.1 and 1.0 l/min. The flow rate used is dependent on the expected concentration of the aldehyde compound to be measured but is typically set at 1 l/min. Other samplers should be prepared in accordance with the manufacturer's instructions. If there is no indication of the concentration it may be appropriate to use backup filters to detect breakthrough. Some commercial samplers have backup sections as an integral part of the design.

29 Set aside a minimum of three unused filters or sorbent tubes as blanks for each set of samples collected. Ensure the blanks are handled in the same way as the samples but without drawing air through them.

30 Attach the sampler to the worker's lapel as close to the mouth and nose as possible. For pumped samplers, place the sampling pump in a convenient pocket or attach it to the worker via a belt or harness. Record the start time and switch on the sampling pump.

31 Check the sampler and pump periodically during sampling to ensure they are still working. If necessary re-measure and adjust the flow rate.

32 At the end of the sampling period, record the end time, measure the flow-rate, switch off the sampling pump and seal the sampler with its protective cover or cap.

33 Transport the sampling media to the laboratory for analysis either in the sampling heads or a suitable container.

34 Filter samples may be stored at room temperature for up to two weeks. Longer storage may be possible depending on the detection limit required and the nature of the sampling medium.

## Sample preparation

35 Transfer the sample filter to a 4 ml glass screw-top HPLC vial.

36 Add 2 ml of acetonitrile, cap and leave to stand for at least 30 minutes with occasional agitation.

37 Commercial sorbent tubes should be desorbed in acetonitrile using the volume specified in the manufacturer's instructions.

38 Treat the blanks in the same way as the samples.

39 Once desorbed the sample solutions may be stored at room temperature for up to four weeks prior to analysis.

## Instrumental conditions

40 HPLC:

| | | |
|---|---|---|
| (a) | Column | Waters Resolve™; 300 mm × 3.9 mm; 5 µm particle size |
| (b) | Mobile phase | 55% acetonitrile + 45% 0.01M $NaH_2PO_4$ in 90:1 acetonitrile: water |
| (c) | Flow rate | 1 ml/min |

| | | |
|---|---|---|
| (d) | Column temperature | 50 °C |
| (e) | Detector wavelength | Summed UV absorption from 290 – 500 nm |
| (f) | Cell pathlength | 10 mm |

Other columns and conditions may also be suitable.

41 Photodiode Array detectors allow peak identification by the UV spectrum. Fixed wavelength instruments, eg 360 nm may be used assuming chromatographic resolution.

42 Where necessary other analytical conditions may be used to avoid interference from other materials.

## Calibration

43 Prepare five calibration standard solutions of a suitable concentration by accurately weighing aliquots of the purified aldehyde derivative (see Appendix 1) into separate 50 ml volumetric flasks and making up to volume with acetonitrile.

44 For formaldehyde samples suitable masses of the derivative are typically 100 µg, 500 µg, 1 mg, 2 mg and 10 mg. Alternatively the two most dilute standards can be prepared by dilution.

45 Analyse the calibration solutions and measure the peak areas of the target aldehyde. Plot the peak areas against the corresponding aldehyde concentration (in µg/ml) and construct the line of best fit. The slope of this line is the detector response factor for the target aldehyde.

46 Modern HPLC equipment is usually sufficiently stable that a new calibration is not required with each set of samples. However, in order 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 below (paragraph 47) or from a sampler spiked with a known quantity of the target analyte.

## Sample analysis

47 Prepare a QA solution by dissolving a known mass of the aldehyde derivative in acetonitrile or by desorbing a sampler treated with a known quantity of aldehyde. Ideally, this should be made from derivative from an independent source.

48 Analyse the samples, blanks and QA solutions and determine the chromatographic peak areas for the target aldehyde.

49 Convert the chromatographic peak area to an aldehyde concentration (in µg/ml) by dividing by the response factor value obtained from the calibration standards.

50 Convert the aldehyde concentration to a mass (in µg) by multiplying by the desorption volume (in ml).

51 Calculate the mean mass of aldehyde (in µg) in the blanks.

52 For each sample calculate a blank corrected mass of aldehyde, Ms, by subtracting the mean mass of aldehyde in the blanks (paragraph 51) from the uncorrected mass (paragraph 50).

53 For samplers with a front and backup section, calculate a total blank corrected mass of aldehyde, Ms, by adding together the blank corrected results from the front and backup sections (paragraph 52).

## Calculation of results

54 Calculate the volume of air sampled for each sample, Vs, in litres.

55 Calculate the analyte concentration in each air sample, C, in mg/m$^3$, using the equation:

$$C = M_s / V_s$$

56 Results may be expressed as the volume fraction in air, in ppm, using the equation:

$$C' = C \times \frac{24.5}{Mw} \times \frac{101}{P} \times \frac{T}{298}$$

where

C′ = volume fraction in air (ppm)
24.5 = molar volume (litres) at 298 K and 101 kPa
Mw = aldehyde molecular weight (g/mol)
T = temperature of sampled air (K)
P = pressure of sampled air (kPa).

## Sources of error

57 Isomeric carbonyl compounds and other materials may interfere with the method due to similar retention times or depletion of the reagent. Interferences caused by similar retention times can usually be avoided by using different chromatographic conditions. Large concentrations of carbonyl compounds such as acetone or of oxidants such as $NO_2$ can reduce the response to formaldehyde by destroying the reagent or

the reaction product. Ozone can also interfere with the method. If the presence of ozone is suspected it can be removed with a denuder or cartridge containing potassium iodide.

58 Sample stability is considered in Appendix 1.

## Appendix 1: Additional Information

1 Significant supplementary information can be found below. Other further information can be found in the back-up data report published online[6].

### Evaluation of the method

#### *Precision*

2 For formaldehyde, the repeatability of the method, measured by desorbing commercial samples spiked from test atmospheres at a concentration of approximately 2.5 mg/m$^3$, was found to be better than 5%.

#### *Overall Uncertainty*

3 The overall uncertainty of the method varies from batch to batch and with sample loading but for formaldehyde is typically less than 10%.

#### *Sampling efficiency*

4 The sampling efficiencies of several samplers were tested using atmospheres of formaldehyde. These tests showed sampling efficiencies for formaldehyde of 88 to 109%.

5 Recovery of the aldehydes from the samplers is typically in excess of 99%.

#### *Detection limits*

6 Detection limits for the analysis will depend on the samplers, the sampling conditions and the details of the equipment used. Tests carried out on several pumped samplers gave detection limits for a 15-minute sample of 0.0005 mg/m$^3$ to 0.003 mg/m$^3$. Similar tests carried out on a commercial diffusive sampler gave a detection limit of 0.13 mg/m$^3$, again based on a 15 minute sample. Longer sampling times give rise to lower detection limits.

#### *Sample stability*

7 Various commercial samplers were spiked with known amounts of formaldehyde. The samples were found to be stable for the times stated by the manufacturers, ie at least 14 days.

#### *Preparation of purified DNPH*

8 Prepare 200 ml of 4.8 M (approx.) hydrochloric acid by adding 80 ml of the concentrated acid to 120 ml of water.

9 Dissolve 6 g of 2,4-DNPH in 100 ml of the 4.8 M hydrochloric acid.

10 Filter the solution through a glass fibre filter and leave to cool, allowing the formation of pale yellow needle-shaped crystals of DNPH hydrochloride.

11 Wash the crystals with acetonitrile followed by diethyl ether and leave to dry.

12 Recrystallise the crystals from 70 ml of 4.8 M hydrochloric acid.

13 Wash and dry the crystals with acetonitrile.

14 Filter off the pure salt and powder it. Add to a solution of 9 ml of 25% w/v ammonia in 50 ml of water with stirring. This converts the DNPH hydrochloride salt back to the free base.

15 Filter off the DNPH free base and wash with water.

16 Recrystallise the DNPH from acetonitrile.

### Preparation of aldehyde derivatives

17 The aldehyde-DNPH derivatives to be used in the calibration of the HPLC analytical system are prepared as follows.

18 Add 2 g of the purified DNPH crystals to 10 ml of concentrated hydrochloric acid.

19 Add 80 ml of ethanol. Warm and stir the mixture to dissolve the DNPH reagent.

20 Mix 0.01 moles of aldehyde with 10 ml of ethanol.

21 Add the aldehyde solution (paragraph 20) to the DNPH reagent solution (paragraph 19), whereupon the aldehyde-DNPH derivative will rapidly form.

22 After cooling, filter off the yellow derivative using a sintered glass filter.

23 Purify the derivative by recrystallisation from boiling acetonitrile.

24 Wash the crystals with ether and dry in air.

25 A number of aldehyde-DNPH standards are commercially available. Analysts should ensure that the

aldehyde derivatives used are of sufficient quality by comparison with certified reference materials and participation in a Quality Assurance scheme.

## References


1 BS EN ISO 16000-3:2003 *Indoor air. Determination of formaldehyde and other carbonyl compounds. Active sampling method* British Standards Institution*

2 *General methods for sampling and gravimetric analysis of respirable and inhalable dust* MDHS14/3 (Third edition) HSE Books 2000 www/hse.gov.uk/pubns/mdhs/

3 BS EN ISO 8655:2002 *Piston-operated volumetric apparatus. Parts 1–6* British Standards Institution *

4 BS EN ISO 3696:1995 *Water for analytical laboratory use. Specification and test methods* British Standards Institution*

5 BS EN 482:1994 *Workplace atmospheres. General requirements for the performance of procedures for the measurement of chemical agents* British Standards Institution *

6 Cuthbert J; *Revision of the MDHS methods for measurement of aldehydes*, Health and Safety Laboratory Report AS/2008/15.


*Amendments may be made occasionally and readers should ensure that they are using the correct edition.

## Further information

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