INTERNATIONAL STANDARD

ISO 16000-3

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Indoor air —

Part 3: Determination of formaldeyhde and other carbonyl compounds — Active sampling method

Air intérieur —

Partie 3: Dosage du formaldéhyde et d'autres composés carbonylés — Méthode par échantillonnage actif

Reference number
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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 16000 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 16000-3 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

ISO 16000 consists of the following parts, under the general title *Indoor air*

— *Part 1: General aspects of sampling strategy*

— *Part 2: Sampling strategy for formaldehyde*

— *Part 3: Determination of formaldeyhde and other carbonyl compounds — Active sampling method*

— *Part 4: Determination of formaldehyde — Diffusive sampling method*

— *Part 6: Determination of volatile organic compounds in indoor and chamber air by active sampling on TENAX TA sorbent, thermal desorption and gas chromatography using MS/FID*

— *Part 7: Sampling strategy for determination of airborne asbestos fibre concentrations*

Annexes A and B of this part of ISO 16000 are for information only.

Introduction

This part of ISO 16000 is intended to be used for characterizing indoor air following the sampling strategy described in ISO 16000-2. It is applicable to formaldehyde and other carbonyl compounds. It has been tested for 14 aldehydes and ketones. Formaldehyde is the simplest carbonyl compound, with one carbon, one oxygen and two hydrogen atoms. In its monomolecular state, it is a colourless, pungent, reactive gas. It has been used in the production of urea-formaldehyde resins, adhesives and insulating foams. Emissions from particle (chip) board and wall insulation are the major sources of formaldehyde in indoor air.

Formaldehyde is collected by passing air through a reactive medium that converts the compound to a derivative of lower vapour pressure that is more efficiently retained by the sampler and can be easily analysed. This part of ISO 16000 determines formaldehyde and other carbonyl compounds by reaction with 2,4-dinitrophenylhydrazine coated onto a sorbent to convert them to their corresponding hydrazones, which can be recovered and measured with high sensitivity, precision and accuracy. Other carbonyl compounds that may be emitted into air from solvents, adhesives, cosmetics, and other sources can also be determined using this part of ISO 16000.

The sampling procedure is based on U.S. EPA method TO-11 A [1].

Formaldehyde and certain other carbonyl compounds have a high toxic potential [2].

Indoor air —

Part 3: Determination of formaldeyhde and other carbonyl compounds — Active sampling method

1 Scope

This part of ISO 16000 describes a procedure for the determination of formaldehyde (HCHO)[1)] and other carbonyl compounds[1)] (aldehydes and ketones) in air. The method is specific for formaldehyde but, with modification, at least thirteen other carbonyl compounds can be detected and quantified. It is suitable for determination of formaldehyde and other carbonyl compounds in the concentration range of approximately 1 µg/m^3 to 1 mg/m^3. The sampling method gives a time-weighted average (TWA) sample. It can be used for long-term (1 h to 24 h) or short-term (5 min to 60 min) sampling of air for formaldehyde.

This part of ISO 16000 describes a sampling and analysis procedure for formaldehyde and other carbonyl compounds that involves collection from air onto cartridges coated with 2,4-dinitrophenylhydrazine (DNPH) and subsequent analysis by high performance liquid chromatography (HPLC) with detection by ultraviolet absorption [1, 3].

The procedures described are written specifically for the sampling and analysis of formaldehyde in air using an adsorbent cartridge and HPLC. The method also permits the determination of other aldehydes and ketones collected from air.

This part of ISO 16000 is applicable to the following carbonyl compounds:

Formaldehyde	Acetaldehyde	Acetone
Benzaldehyde	Butyraldehyde	Valeraldehyde
2,5-Dimethylbenzaldehyde		Crotonaldehyde
Isovaleraldehyde	Propionaldehyde	Hexanal
o-Tolualdehyde	*p*-Tolualdehyde	*m*-Tolualdehyde

1) Instead of the nomenclature according to IUPAC regulations, the more common names are used in this International Standard:

formaldehyde: methanal
acetaldehyde: ethanal
acetone: 2-propanone
butyraldehyde: butanal
crotonaldehyde: 2-butenal
isovaleraldehyde: 3-methylbutanal
propionaldehyde: propanal
m-tolualdehyde: 3-methylbenzaldehyde
o-tolualdehyde: 2-methylbenzaldehyde
p-tolualdehyde: 4-methylbenzaldehyde
valeraldehyde: pentanal

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 16000. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 16000 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 9000-1:1994; *Quality management and quality assurance standards — Part 1: Guidelines for selection and use.*

ISO 9000-2:1997; *Quality management and quality assurance standards — Part 2: Generic guidelines for the application of ISO 9001, ISO 9002 and ISO 9003.*

ISO 16000-1, *Indoor air — Part 1: General aspects of sampling strategy.*

ISO 16000-2, *Indoor air — Part 2: Sampling strategy for formaldehyde.*

ISO 16000-4, *Indoor air — Part 4: Determination of formaldehyde — Diffusive sampling method.*

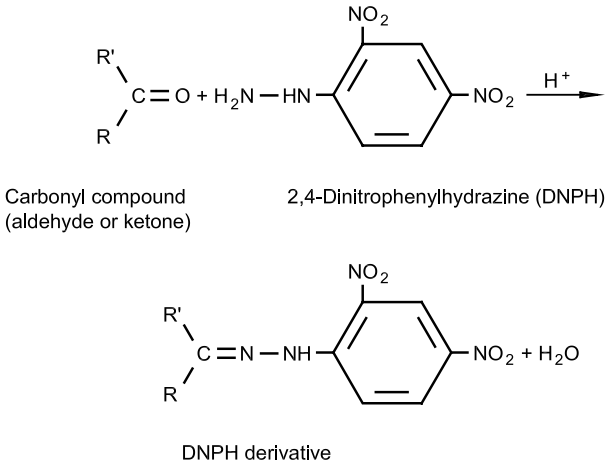
ISO 17025:1999; *General requirements for the competence of testing and calibration laboratories.*

EN 45001:1989; *General criteria for the operation of testing laboratories.*

3 Principle

This part of ISO 16000 involves drawing air through a cartridge containing silica gel coated with 2,4-dinitrophenylhydrazine (DNPH) reagent. The principle of the method is based on the specific reaction of carbonyl group with DNPH in the presence of an acid to form stable derivatives according to the reaction shown in Figure 1. The DNPH derivatives are analysed for the parent aldehydes and ketones utilizing high performance liquid chromatography (HPLC) with UV detection or diode array detection. The detection has been extended to other carbonyl compounds that can be determined as outlined in 9.3.5.

This part of ISO 16000 instructs the user on how to prepare sampling cartridges from commercially available chromatographic grade silica gel cartridges by the application of acidified DNPH to each cartridge. Alternatively, pre-coated DNPH silica gel cartridges are available and are recommended since they are generally more uniform in manufacture and possess lower blank levels. However, if commercial cartridges are used they shall be demonstrated to meet the performance criteria of this part of ISO 16000. Another advantage of commercial cartridges is that they are available with larger particle-size silica gel that results in a lower pressure-drop across the cartridge. These low pressure-drop cartridges may be more suitable for sampling air using battery-powered personal sampling pumps.



Key

R alkyl or aromatic groups for ketones, or H for aldehydes

R′ alkyl or aromatic groups, for ketones

Figure 1 — Reaction of carbonyl compounds

4 Limitations and interferences

4.1 General

The sampling flowrate, as described in this part of ISO 16000, has been validated for sampling rates up to 1,5 l/min. This flowrate limitation is principally due to the high pressure drop (> 8 kPa at 1,0 l/min) across the user-prepared silica gel cartridges, which have particle sizes of 55 µm to 105 µm. These cartridges are not generally compatible with battery-powered pumps used in personal sampling equipment (e.g. those used by industrial hygienists).

The solid-sorbent sampling procedure is specific for sampling and analysis of formaldehyde. Interferences in this method are caused by certain isomeric aldehydes or ketones that may be unresolved by the HPLC system when analysing for other aldehydes and ketones. Organic compounds that have the same retention times and significant absorbance at 360 nm as the DNPH derivative of formaldehyde will interfere. Such interferences can often be overcome by altering the separation conditions (for example, using alternative HPLC columns or mobile phase compositions).

Formaldehyde contamination of the DNPH reagent is a frequently encountered problem. The DNPH shall be purified by multiple recrystallizations in UV-grade acetonitrile. Recrystallization is accomplished, at 40 °C to 60 °C, by slow evaporation of the solvent to maximize crystal size. Impurity levels of carbonyl compounds in the DNPH are determined prior to use by HPLC and should be less than 0,15 µg per cartridge.

Exposure of the DNPH-coated sampling cartridges to direct sunlight may produce artefacts and should be avoided [4].

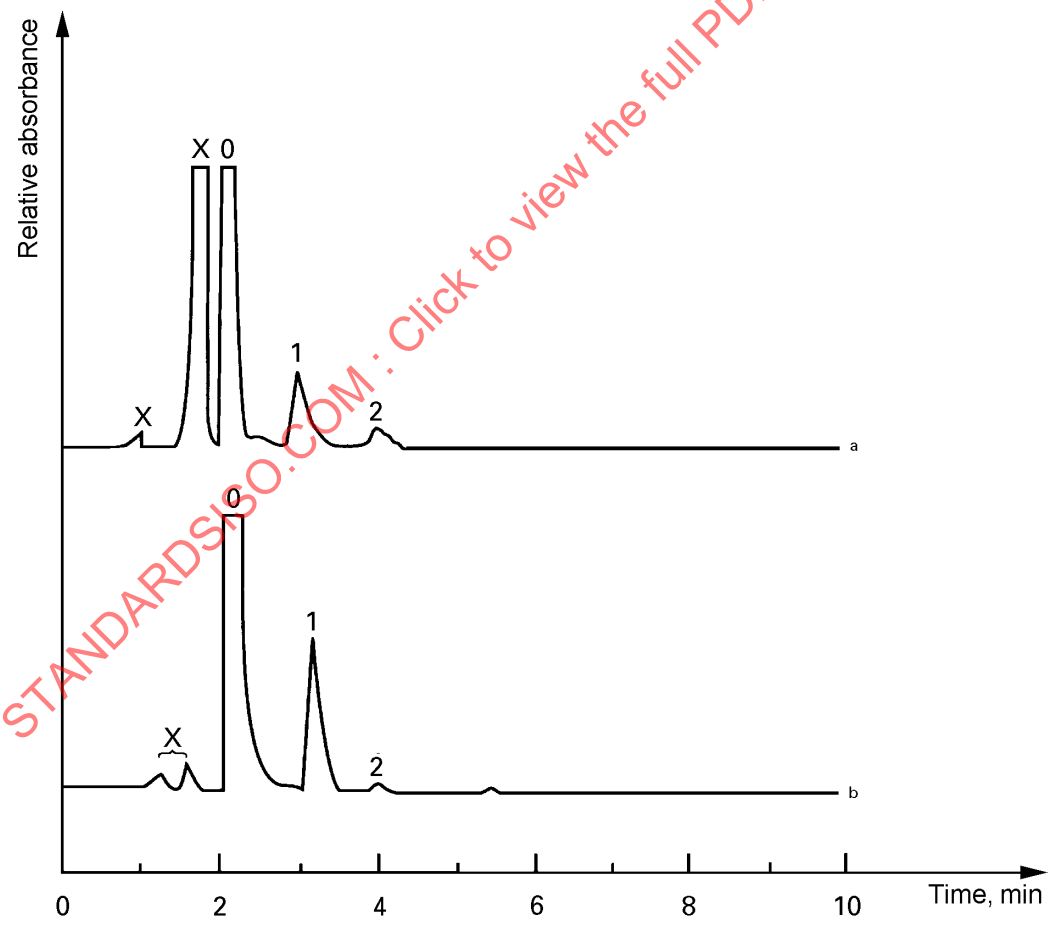
This method cannot be used for accurate quantification of acrolein in air. Inaccurate results for acrolein may result from the formation of multiple derivative peaks and the instability of the peak ratios [9].

NO_2 reacts with DNPH. High concentrations of NO_2 (for example, for gas cooking stoves) may cause problems as the retention time of the DNPH derivative may be similar to that of the DNPH formaldehyde derivative, depending on the HPLC column and the parameters [14, 15, 16].

4.2 Ozone interference

If there is suspicion that abnormally high levels of ozone may be present in the area being sampled (e.g. from office copiers), special care should be exercised. Ozone has been shown to interfere negatively by reacting with both DNPH and its derivatives (hydrazones) in the cartridge [5]. The extent of interference depends on the temporal variations of both the ozone and the carbonyl compounds and the duration of sampling. Significant negative interference from ozone has been observed even at concentrations of formaldehyde and ozone typical of clean ambient air (2 µg/m^3 and 80 µg/m^3, respectively) [6]. The presence of ozone in the sample is readily inferred upon analysis by the appearance of new compounds with retention times shorter than that of the hydrazone of formaldehyde. Figure 2 shows chromatograms of samples of a formaldehyde-spiked air stream with and without ozone.

The most direct solution to ozone interference is to remove the ozone before the sampled air reaches the cartridge. This may be accomplished by the use of an ozone denuder or scrubber placed in front of the cartridge. Both ozone denuders and scrubber cartridges are commercially available. A denuder may be constructed of 1 m of 0,64 cm outside diameter by 0,46-cm inside diameter copper tubing, that is filled with a saturated solution of potassium iodide in water, allowed to stand for a few minutes (e.g. 5 min), drained and dried with a stream of clean air or nitrogen for about 1 h. The capacity of the ozone denuder as described is about 200 µg ozone/m^3 h. Test aldehydes (formaldehyde, acetaldehyde, propionaldehyde, benzaldehyde and *p*-tolualdehyde) that were dynamically spiked into an ambient sample air stream passed through the ozone denuder with practically no losses [7]. Commercial ozone scrubbers made from a cartridge filled with 300 mg to 500 mg of granular potassium iodide have also been found to be effective in removing ozone [8].



Key

X	unknown	2	Acetaldehyde
0	DNPH	a	With ozone
1	Formaldehyde	b	Without ozone

Figure 2 — Cartridge samples of formaldehyde in an air stream with and without ozone

5 Safety measures

5.1 This part of ISO 16000 does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this part of ISO 16000 to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

5.2 2,4-Dinitrophenylhydrazine is explosive in the dry state and shall be handled with extreme care. It is also toxic (LD_{50}, rat = 654 mg/kg), has been shown to be mutagenic in some tests, and is irritating to the eyes and skin.

5.3 Perchloric acid at concentrations less than 68 % mass fraction is stable and non-oxidizing at room temperature. However, it is readily dehydrated at temperatures above 160 °C and can cause explosions on contact with alcohols, wood, cellulose and other oxidizable materials. It should be stored in a cool, dry place and used only in a chemical fume hood with caution.

6 Apparatus

Ordinary laboratory apparatus and the following.

6.1 Sampling

6.1.1 Sampling cartridge, packed with silica gel and coated with DNPH in accordance with clause 8, or as available commercially.

The cartridge shall contain a minimum quantity of 350 mg of silica gel with a minimum DNPH loading of 0,29 % mass fraction. The ratio of the silica gel bed diameter to bed length shall not exceed 1:1. The capacity of the cartridge for formaldehyde shall be at least 75 µg and the collection efficiency at least 95 % at a sampling rate of 1,5 l/min. Sampling cartridges with very low blank levels and high performance are commercially available.

NOTE A pressure drop through the user-prepared sample cartridge of about 19 kPa at a sampling rate of 1,5 l/min has been observed. Some commercially available pre-coated cartridges may exhibit lower pressure-drops, which will permit the use of battery-operated personal sampling pumps.

6.1.2 Air sampling pump, capable of accurately and precisely sampling at a flowrate of 0,1 l/min to 1,5 l/min.

6.1.3 Flow controller, mass flowmeters and mass flow controllers, or other suitable device for metering/setting air flowrate of 0,50 l/min to 1,20 l/min through sample cartridge.

6.1.4 Flow calibrator, such as a rotameter, soap-bubble meter or wet test meter.

6.2 Sample preparation

6.2.1 Cartridge containers, e.g. borosilicate glass culture tubes (20 mm by 125 mm) with polypropylene screw caps, or other suitable containers, to transport coated cartridges.

6.2.2 Polyethylene gloves to handle silica gel cartridges.

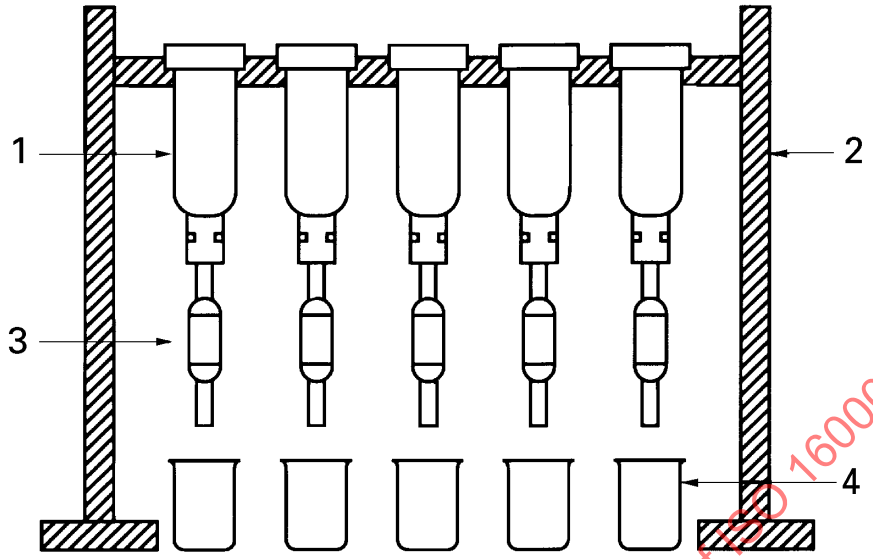
6.2.3 Transportation containers, friction-top metal cans (e.g. of volume 4 l) or other suitable containers, with polyethylene air-bubble packing or other suitable padding, to hold and cushion the sealed cartridge containers.

NOTE A heat-sealable foil-lined plastic pouch of the type included with some commercial pre-coated DNPH cartridges may be used for storing a DNPH-coated cartridge after sampling, if appropriate.

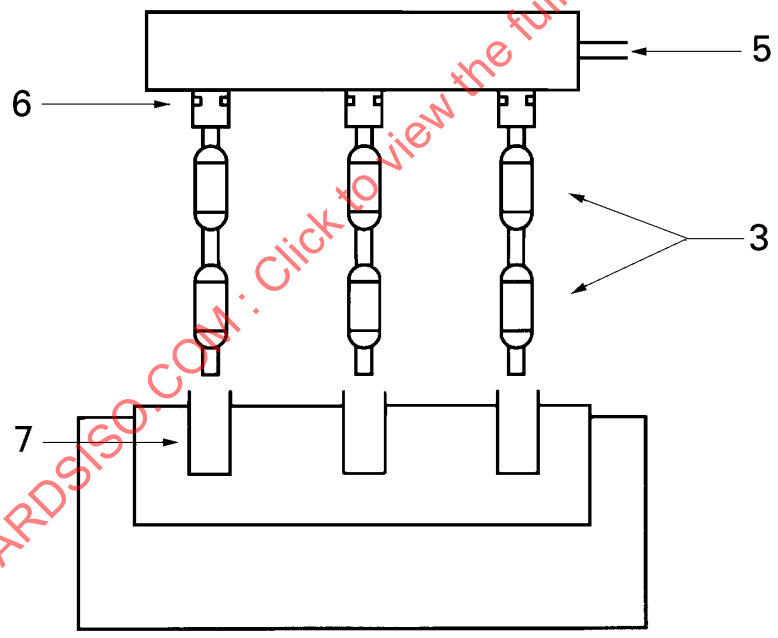
6.2.4 Support for coating cartridges.

A syringe rack, made from an aluminium plate (0,16 cm × 36 cm × 53 cm) with adjustable legs on four corners. A matrix (5 × 9) of circular holes of diameter slightly larger than the diameter of the 10 ml syringes, symmetrically drilled from the centre of the plate, to enable batch processing of 45 cartridges for cleaning, coating and/or sample elution (see Figure 3).

6.2.5 Cartridge-drying manifold, such as a support with gas connectors and with multiple standard male syringe connectors (see Figure 3).



a) Rack for coating cartridges



b) Rack for drying DNPH-Coated cartridges

Key

1 10 ml glass syringes
2 Test tube rack
3 Cartridges
4 Waste beakers
5 N_2 gas stream
6 Syringe fitting
7 Waste vials

Figure 3 — Syringe rack for coating and drying sample cartridges

NOTE The apparatus described in 6.2.4 and 6.2.5 are needed only if the user chooses to make his own DNPH-coated cartridges.

6.3 Sample analysis

6.3.1 HPLC system, consisting of a mobile phase reservoir; a high-pressure pump; an injection valve (automatic sampler with a 25 µl or other convenient loop volume); a C18 reverse phase (RP) column (for example 25 cm × 4,6 mm inside diameter, 5 µm particle size); a UV detector or diode array detector operating at 360 nm; and a data system or strip chart recorder.

The DNPH-formaldehyde derivative is determined using isocratic reverse phase HPLC, equipped with an ultraviolet (UV) absorption detector operated at 360 nm. A blank cartridge is likewise desorbed and analysed. Formaldehyde and other carbonyl compounds in the sample are identified and quantified by comparison of their retention times and peak heights or peak areas with those of standard solutions.

NOTE Most commercial HPLC analytical systems are adequate for this application.

6.3.2 Syringes and pipettes

6.3.2.1 HPLC injection syringes, with capacity at least four times the loop volume (see 6.3.1).

6.3.2.2 Syringes of volume 10 ml, used to prepare DNPH-coated cartridges (polypropylene syringes are adequate).

6.3.2.3 Syringe fittings and plugs, to connect cartridges to the sampling system and to cap prepared cartridges.

6.3.2.4 Pipettes, positive-displacement, repetitive-dispensing, with capacities in the 0 ml to 10 ml range.

7 Reagents

7.1 2,4-Dinitrophenylhydrazine, recrystallized at least twice with UV-grade acetonitrile before use.

7.2 Acetonitrile, UV-grade (each batch of solvent should be tested before use).

7.3 Perchloric acid, 60 % mass fraction, $\rho = 1{,}51$ kg/l.

7.4 Hydrochloric acid, 36,5 % to 38 % mass fraction, $\rho = 1{,}19$ kg/l.

7.5 Formaldehyde, 37 % solution (mass fraction).

7.6 Aldehydes and ketones, high purity, used for preparation of DNPH derivative standards (optional).

7.7 Ethanol or methanol, HPLC grade

7.8 Nitrogen, high purity grade (best source).

7.9 Charcoal, granular (best source).

7.10 Helium, high purity grade (best source).

8 Preparation of reagents and cartridges

8.1 Purification of 2,4-dinitrophenylhydrazine

Formaldehyde contamination of the DNPH reagent is a frequently encountered problem. The DNPH shall be purified by multiple recrystallizations in UV-grade acetonitrile. Recrystallization is accomplished, at 40 °C to 60 °C, by slow evaporation of the solvent to maximize crystal size. Impurity levels of carbonyl compounds in the DNPH are determined prior to use by HPLC and should be less than 0,15 µg per cartridge and per individual compound.

Prepare a supersaturated solution of DNPH by boiling excess DNPH in 200 ml of acetonitrile for approximately 1 h. After 1 h, remove and transfer the supernatant to a covered beaker on a hot plate and allow gradual cooling to 40 °C to 60 °C. Maintain the solution at this temperature (40 °C) until 95 % volume fraction of solvent has evaporated. Decant the solution to waste, and rinse the remaining crystals twice with three times their apparent volume of acetonitrile. Transfer the crystals to another clean beaker, add 200 ml of acetonitrile, heat to boiling, and again let crystals grow slowly at 40 °C to 60 °C until 95 % volume fraction of the solvent has evaporated. Repeat the rinsing process as described above. Take an aliquot of the second rinse, dilute ten times with acetonitrile, acidify with 1 ml of perchloric acid (3,8 mol/l) per 100 ml of DNPH solution, and analyse by HPLC, in accordance with 9.3.4.

WARNING — Carry out this procedure under a properly ventilated hood and behind an explosion shield.

NOTE An acid is necessary to catalyse the reaction of the carbonyl compounds with DNPH. Most strong inorganic acids such as hydrochloric, sulfuric, phosphoric or perchloric acids will perform satisfactorily. In some rare cases, hydrochloric and sulfuric acids may cause problems.

An acceptable impurity level is < 0,025 µg/ml of formaldehyde hydrazone in recrystallized DNPH reagent or 0,02 % mass fraction of the DNPH.

If the impurity level is not acceptable for the intended sampling application, repeat recrystallization. Transfer the purified crystals to an all-glass reagent bottle, add 200 ml of acetonitrile, stopper, shake gently, and let stand overnight. Analyse the supernatant by HPLC according to 9.3.4. If the impurity level is not satisfactory, pipette off the solution to waste, then add 25 ml of acetonitrile to the purified crystals. Repeat rinsing with 20 ml portions of acetonitrile until a satisfactorily low impurity level in the supernatant is confirmed by HPLC analysis.

If the impurity level is satisfactory, add another 25 ml of acetonitrile, stopper, and shake the reagent bottle, then set aside. The saturated solution above the purified crystals is the stock DNPH reagent. Maintain only a minimum volume of saturated solution adequate for day-to-day operation. This will minimize waste of purified reagent, should it be necessary to re-rinse the crystals to decrease the level of impurity for applications requiring more stringent purity specifications. Use clean pipettes when removing saturated DNPH stock solution for any analytical applications. Do not pour the stock solution from the reagent bottle.

8.2 Preparation of DNPH-formaldehyde derivative

To a portion of the recrystallized DNPH add sufficient HCl (2 mol/l) to obtain an approximately saturated solution. Add to this solution formaldehyde in molar excess of the DNPH. Filter the DNPH-formaldehyde precipitate, wash it with HCl (2 mol/l) and water, and allow it to dry in air.

Check the purity of the DNPH-formaldehyde derivative by melting point determination (165 °C to 166 °C) or HPLC analysis. If the impurity level is not acceptable, recrystallize the derivative in ethanol. Repeat the purity check and recrystallization as necessary until an acceptable level of purity (for example, 99 % mass fraction) is achieved.

The DNPH-formaldehyde derivative should be stored under refrigeration (4 °C) and protection from light. It should be stable for at least six months. Storage under nitrogen or argon further prolongs the lifetime of the derivative.

Melting points of DNPH derivatives of several carbonyl compounds are given in annex B.

DNPH derivatives of formaldehyde and other carbonyls suitable for use as standards are commercially available both in the form of pure crystals and as individual or mixed stock solutions in acetonitrile.

8.3 Preparation of DNPH-formaldehyde standards

Prepare a standard stock solution of the DNPH-formaldehyde derivative by dissolving accurately weighed amounts in acetonitrile. Prepare a working calibration standard mix from the standard stock solution. The concentration of the DNPH-formaldehyde derivative in the standard mix solutions should be adjusted to reflect the range of concentrations expected in real samples.

Individual stock solutions of approximately 100 mg/l can be prepared by dissolving 10 mg of the solid derivative in 100 ml of acetonitrile. The individual solution is used to prepare calibration standards containing the derivative of interest at concentrations of 0,5 µg/ml to 20 µg/ml, that spans the concentration of interest.

Store all standard solutions in tightly capped containers in a refrigerator and protected from light. Allow them to equilibrate to room temperature before use. They should be replaced after four weeks.

8.4 Preparation of DNPH-coated silica gel cartridges

8.4.1 General

This procedure shall be performed in an atmosphere with a very low aldehyde background. All glassware and plasticware shall be thoroughly cleaned and rinsed with deionized water and aldehyde-free acetonitrile. Contact of reagents with laboratory air shall be minimized. Polyethylene gloves shall be worn when handling the cartridges.

8.4.2 DNPH coating solution

Pipette 30 ml of saturated DNPH stock solution into a 1 000 ml volumetric flask, then add 500 ml acetonitrile. Acidify with 1,0 ml of concentrated HCl.

The atmosphere above the acidified solution should preferably be filtered through a DNPH-coated silica gel cartridge, to minimize contamination from laboratory air. Shake solution, then make up to volume with acetonitrile. Stopper the flask, invert, and shake several times until the solution is homogeneous. Transfer the acidified solution to a reagent bottle equipped with a positive-displacement dispenser of capacity in the 0 ml to 10 ml range.

Prime the dispenser and slowly dispense 10 ml to 20 ml to waste. Dispense an aliquot solution to a sample vial, and check the impurity level of the acidified solution by HPLC according to 9.3.4. The impurity level should be $< 0{,}025$ µg formaldehyde/ml.

8.4.3 Coating of silica gel cartridges

Open the cartridge package, connect the short end to a 10-ml syringe, and place it in the syringe rack as illustrated in Figure 3 a) and b). Using a positive-displacement repetitive pipette, add 10 ml of acetonitrile to each of the syringes. Let liquid drain to waste by gravity.

Remove any air bubbles that may be trapped between the syringe and the silica cartridge by displacing them with the acetonitrile in the syringe.

Set the repetitive dispenser, containing the acidified DNPH coating solution, to dispense 7 ml into the cartridges. Once the effluent flow at the outlet of the cartridge has stopped, dispense 7 ml of the coating reagent into each of the syringes. Let the coating reagent drain by gravity through the cartridge until flow at the other end of the cartridge stops. Wipe away the excess liquid at the outlet of each of the cartridges with clean tissue paper.

Assemble a drying manifold as shown in Figure 3 b). This contains a previously prepared DNPH-coated cartridge at each of the exit ports (for example, scrubber or “guard cartridges.” These “guard cartridges” serve to remove traces of formaldehyde that may be present in the nitrogen gas supply. They can be prepared by drying a few of the newly coated cartridges in accordance with the instructions below and “sacrificing” these few to ensure the purity of the rest):

Insert cartridge connectors (flared at both ends, 0,64 cm by 2,5 cm outside diameter TFE-fluorocarbon tubing with inside diameter slightly smaller than the outside diameter of the cartridge port) onto the long end of the scrubber cartridges.

Remove the cartridges from the syringes and connect the short ends of the cartridges to the open end of the cartridge connectors already attached to the scrubber cartridges.

Pass nitrogen through each of the cartridges at about 300 ml/min to 400 ml/min. Rinse the exterior surfaces and outlet end of the cartridges with acetonitrile using a Pasteur pipette. After 15 min, stop the flow of nitrogen, wipe the

cartridge exterior free of rinse acetonitrile and remove the dried cartridges. Plug both ends of the coated cartridge with standard polypropylene male syringe plugs and place the plugged cartridge in a borosilicate glass culture tube with polypropylene screw caps.

Put a serial number and a lot number label on each of the individual cartridge glass storage containers and refrigerate the prepared lot until use.

Sampling cartridges have been found to be stable for at least six months when stored at 4 °C in the absence of light.

9 Procedure

9.1 Sample collection

Assemble the sampling system, and ensure that the pump is capable of constant flowrate throughout the sampling period. The sampling cartridges can be safely used for sampling air when the temperature is above 10 °C. If required, add an ozone denuder or scrubber (see 4.2).

Before sample collection, check the system for leaks. Plug the inlet (short end) of the cartridge so no flow is indicated at the outlet end of the pump. The flowmeter should not indicate any air flow through the sampling apparatus.

For unattended or extended sampling periods, a mass flow controller or, as appropriate, a compensated personal sampling pump, is highly recommended to maintain constant flow. The flow controller should be set at least 20 % below the fixed maximum air flowrate through the cartridge.

NOTE 1 The silica gel is held in the cartridge between two fine-porosity filter frits. Air flow during sampling could change as airborne particulates deposit on the front frit. The flow change could be significant when sampling particulate-laden atmospheres.

Install the entire assembly (including a “dummy” sampling cartridge) and check the flowrate at a value near the desired rate. In general, flowrates of 0,5 l/min to 1,2 l/min should be employed. The total moles of carbonyl in the volume of air sampled should not exceed that of the DNPH (2 mg or 0,01 mol/cartridge; 1 mg to 2 mg/cartridge for commercially available pre-coated cartridges). In general, a safe estimate of the sample size should be lower than 75 % of the DNPH mass loading of the cartridge [100 µg to 200 µg as HCHO; with respect to interferences to be taken into account (see clause 4)]. Generally, calibration can be accomplished using a soap-bubble flowmeter or calibrated wet test meter connected to the flow exit, assuming the system is leaktight.

NOTE 2 EN 1232:1997 [13] describes an appropriate calibration scheme that does not require a sealed flow system downstream of the pump.

Measure and record the sampling flowrate at the beginning and end of the sampling period to determine sample volume. If the sampling period exceeds 2 h, the flowrate should be measured at intermediate points during the sampling period. Include a rotameter to allow observation of the flowrate without interruption of the sampling process. Alternatively, a sampling pump which directly measures and continuously records the flowrate can be used.

Before sampling, remove the cartridge container from the friction-top metal can or other suitable container. Let the cartridge warm to room temperature in the glass tube before connecting it to the sampling train.

With a commercial pre-coated DNPH cartridge, also let the cartridge warm to room temperature before connecting to the sampling train.

Using polyethylene gloves, remove the syringe plugs and connect the cartridge to the sampling system with a syringe adapter fitting. Connect the cartridge to the sampling train so that the short end becomes the sample inlet.

With commercial pre-coated DNPH cartridges, follow the manufacturer’s instructions. Some commercial cartridges may be sealed-glass tubes. For these, break the ends of the cartridge with a tube breaker. Connect the cartridge by

inserting the end with the smaller quantity of sorbent to the sampling train so that the larger quantity of sorbent is at the air inlet. Use care when handling the broken ends.

Turn the sampler on and adjust the flow to the desired rate. A typical flowrate through one cartridge is 1,0 l/min and 0,8 l/min for two cartridges in tandem. Operate the sampler for the desired period, with periodic recording of the sampling variables.

If the ambient air temperature during sampling is below 10 °C, the sampling cartridge should be kept in a warmer environment. No significant effects of relative humidity have been observed for sampling under various weather conditions — cold, wet and dry winter months and hot and humid summer months.

At the end of the sampling period, stop the flow. Check the flowrate just before stopping the flow. If the flowrates at the beginning and end of the sampling period differ by more than 15 %, the sample should be marked as suspect.

Immediately after sampling, remove the cartridge (using polyethylene gloves) from the sampling system, cap with the original end plugs, and place it back in the original labelled container. Seal with fluorocarbon tape, and place in a friction-top can containing 2 cm to 5 cm depth of granular charcoal or in another suitable container with appropriate padding. If appropriate, a heat-sealable foil-lined plastic pouch may be used for storing the exposed cartridge. Refrigerate the exposed sample cartridge until analysis. The refrigeration period prior to analysis should not exceed 30 days.

If samples are to be transported to a central laboratory for analysis, the duration of the non-refrigerated period should be kept to a minimum, preferably less than two days.

Calculate the average sample flowrate from the following equation:

$$q_A = \left[q_1 + q_2 + \ldots + q_n\right] / n \qquad (1)$$

where

q_A is the average flowrate, in millilitres per minute;

q_1, q_2, ... q_n are the flowrates determined at beginning, end and intermediate points during sampling;

n is the number of points averaged.

The total flow is then calculated using the following equation:

$$V_m = \left[(T_2 - T_1) \cdot q_A\right] / 1000 \qquad (2)$$

where

V_m is the total volume, in litres, sampled at the measured temperature and pressure;

T_2 is the stop time;

T_1 is the start time;

$T_2 - T_1$ is the total sampling time, in minutes;

q_A is the average flowrate, in millilitres per minute.

9.2 Process blanks

At least one field blank shall be analysed with each set of samples. For sample sets larger than 10 to 20 samples, at least 10 % of the samples analysed shall be field blanks. The number of samples within a group or time frame, or

both, should be recorded so that a specified percentage of blanks is obtained for a given number of air samples. The field blank is treated identically as the samples except that no air is drawn through the cartridge. The performance criteria described in 9.1 should be met for process blanks. It is desirable to analyse blank cartridges retained in the laboratory (lab blanks) as well, to distinguish between possible field and laboratory contamination.

9.3 Sample analysis

9.3.1 Sample preparation

Return the samples to the laboratory in a suitable external container with 2 cm to 5 cm of granular charcoal and store them in a refrigerator until analysis. Alternatively, the samples may also be stored alone in their individual containers. The time between sampling and analysis should not exceed 30 days.

9.3.2 Sample desorption

Connect the sample cartridge (inlet or short end during sampling) to a clean syringe.

The liquid flow during desorption should be in the same direction as the air flow during sampling, to prevent insoluble particulates from getting into the eluate. Reverse desorption may be performed if the eluate is filtered prior to HPLC analysis. A filtered blank extract shall be analysed with each batch of samples to confirm that no contamination is being introduced by the filter.

Place the cartridge/syringe in the syringe rack. Desorb the DNPH derivatives of the carbonyls and the unreacted DNPH from the cartridge (gravity feed) by passing 5 ml of acetonitrile from the syringe through the cartridge to a graduated test tube or to a 5-ml volumetric flask. Other volumes of acetonitrile may be appropriate, depending on the sampling cartridge used.

NOTE A dry cartridge has an acetonitrile holdup volume slightly greater than 1 ml. The eluate flow may stop before the acetonitrile in the syringe is completely drained into the cartridge because of air trapped between the cartridge filter and the syringe adapter tip. If this happens, displace the trapped air with the acetonitrile in the syringe using a long-tip disposable Pasteur pipette.

Dilute to the 5-ml mark with acetonitrile. Label the flask with sample identification. Pipette an aliquot into a sample vial with a fluorocarbon-lined septum. Analyse the aliquot for the carbonyl derivatives by HPLC. As a backup, a second aliquot may be taken and stored under refrigeration until the results of the analysis of the first aliquot are complete and validated. The second aliquot can be used for confirmatory analysis, if necessary.

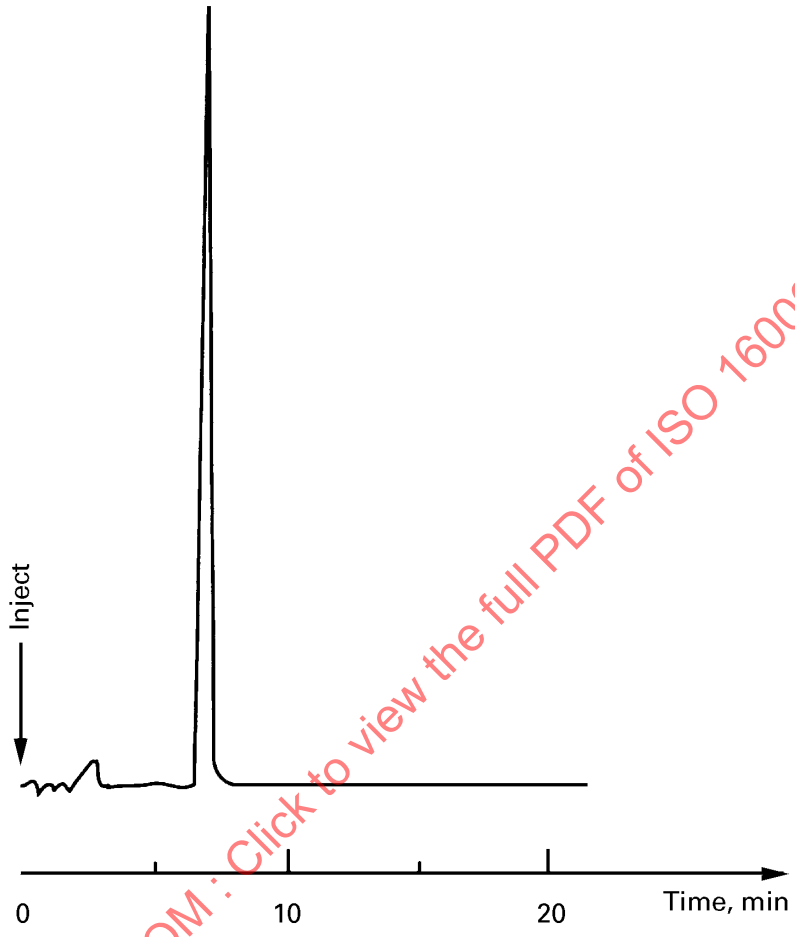
For glass-sealed DNPH sampling tubes that contain two sorbent beds, uncap the end of the tube closest to the second sorbent layer (exit end). Carefully remove the spring and plug of glass wool holding the sorbent layer in place. Empty the sorbent into a clean 4-ml glass vial with a fluorocarbon-lined septum or cap. Mark this as the back-up sampling section. Carefully remove the next plug of glass wool and empty the remaining sorbent into another 4 ml vial. Mark this as the primary sampling section. To each vial, carefully pipette 3 ml acetonitrile into each vial, cap the vials and let stand for 30 min with occasional agitation.

9.3.3 HPLC calibration

Prepare calibration standards in acetonitrile from the DNPH-formaldehyde derivative (see 8.3). Individual stock solutions of 100 mg/l are prepared by dissolving 10 mg of solid derivative in 100 ml of mobile phase.

Analyse each calibration standard (at least five levels) twice and tabulate area response against mass injected (or, more conveniently, versus the DNPH-formaldehyde injected, for a fixed loop volume; see Figures 4 and 5). Perform all calibration runs as described for sample analysis in 9.3.4. To avoid carryover effects, start with the lower concentration. Using the UV detector or the diode array detector, a linear response range of approximately 0,05 µg/ml to 20 µg/ml should be achieved for 25 µl injection volumes. The results can be used to prepare a calibration curve, as illustrated in Figure 6. Linear response is indicated where a correlation coefficient of at least 0,999 for a linear least-squares fit of the data (concentration versus area response) is obtained. The retention times for each analyte should agree within 2 %.

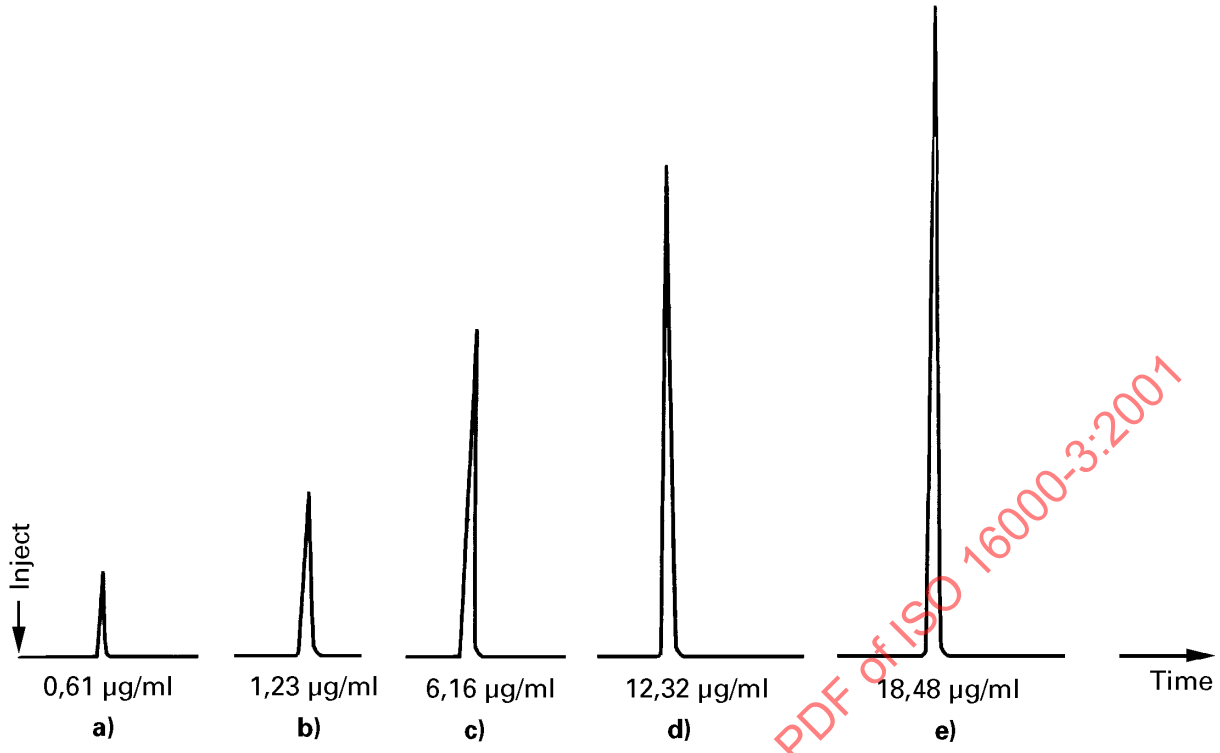
Once linear response has been documented, an intermediate concentration standard near the anticipated levels of each component, but at least ten times the detection limit, should be chosen for daily calibration. The day-to-day response for the various components should be within 10 % for analyte concentrations of 1 µg/ml or greater, and below 20 % for analyte concentrations near 0,5 µg/ml. If greater variability is observed, recalibration may be required or a new calibration curve shall be developed from fresh standards.



Operating parameters, HPLC

Column: C-18 reverse phase
Mobile phase: 60 % acetonitrile/40 % water
Detector: ultraviolet, at 360 nm
Flowrate: 1 ml/min
Retention time: *ca.* 7 min for formaldehyde
Sample injection volume: 25 µl

Figure 4 — Example chromatogram of DNPH-formaldehyde derivative

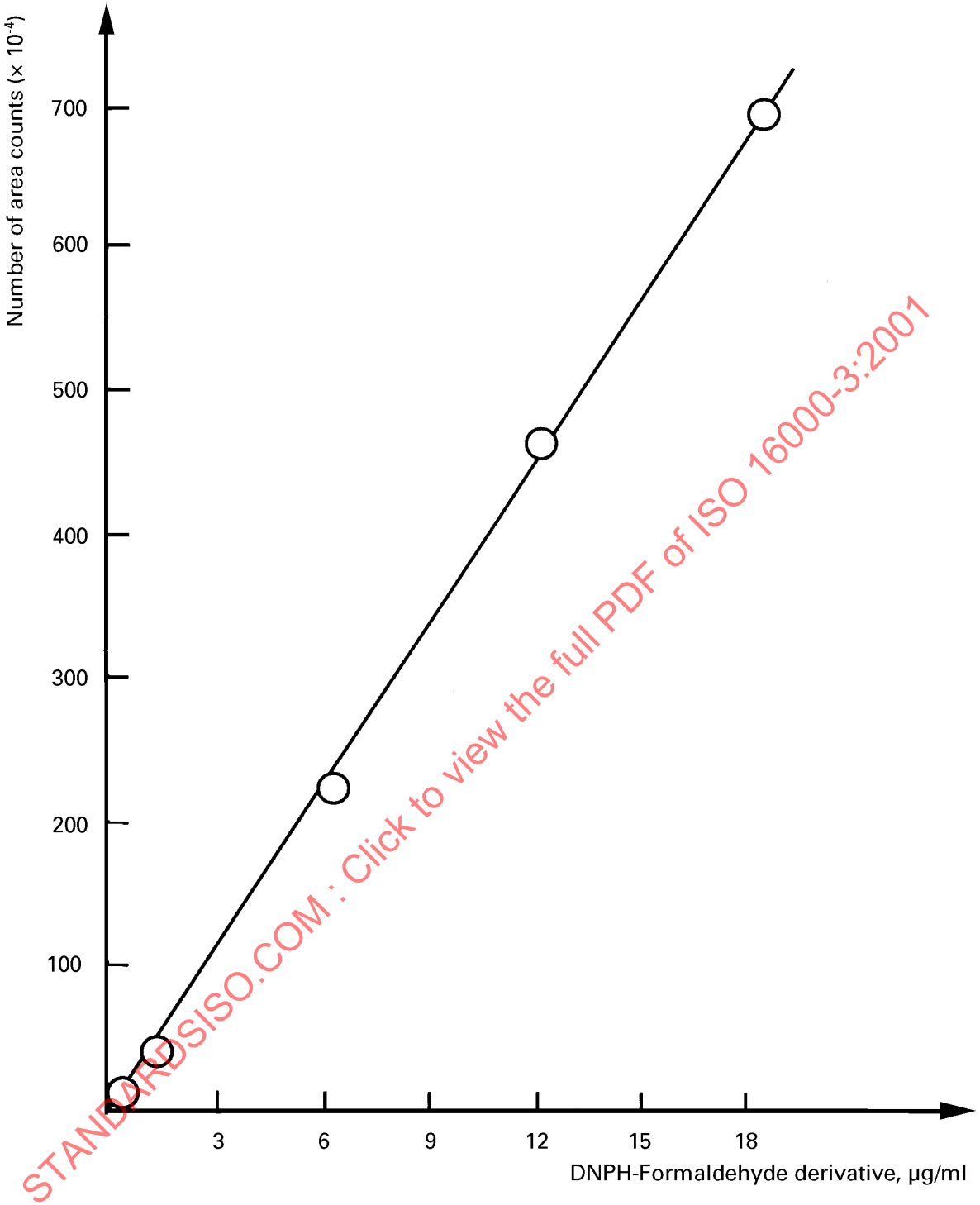


Operating parameters, HPLC

Column: C-18 reverse phase
Mobile phase: 60 % acetonitrile/40 % water
Detector: ultraviolet, at 360 nm
Flowrate: 1 ml/min
Retention time: *ca.* 7 min for formaldehyde
Sample injection volume: 25 µl

Concentration	Number of area counts
0,61 µg/ml	226 541
1,23 µg/ml	452 166
6,16 µg/ml	2 257 271
12,32 µg/ml	4 711 408
18,48 µg/ml	6 953 812

Figure 5 — Examples of HPLC chromatograms of varying concentrations of DNPH-formaldehyde derivative



Operating parameters, HPLC

Correlation coefficient: 0,999 9
Column: C-18 reverse phase
Mobile phase: 60 % acetonitrile/40 % water
Detector: ultraviolet, at 360 nm
Flowrate: 1ml min^{-1}
Retention time: *ca.* 7 min for formaldehyde
Sample injection volume: 25 µl

Figure 6 — Example calibration curve for formaldehyde

9.3.4 HPLC analysis for formaldehyde

Assemble the HPLC system and calibrate as described in 9.3.3. Typical operating parameters are as follows:

Column	C18 (4,6-mm inside diameter × 25 cm, or equivalent); temperature control for the column is not necessary
Mobile phase	60 % acetonitrile/40 % water (volume fraction), isocratic
Detector	Ultraviolet, operating at 360 nm
Flowrate	1,0 ml/min
Retention time	7 min for formaldehyde with one C18 column, 13 min for formaldehyde with two C18 columns
Sample injection volume	25 µl

Before each analysis, check the detector baseline to ensure stable conditions.

Prepare the HPLC mobile phase by mixing 600 ml of acetonitrile and 400 ml of water or set the parameters on the gradient elution HPLC appropriately. Filter this mixture through a 0,22-µm polyester membrane filter in an all-glass and fluorocarbon suction filtration apparatus. Degas the filtered mobile phase by purging with helium for 10 min to 15 min (100 ml/min) or by heating to 60 °C for 5 min to 10 min in an Erlenmeyer flask covered with a watch glass. A constant back-pressure restrictor (350 kPa) or short length (15 cm to 30 cm) of 0,25 mm inside diameter fluorocarbon tubing should be placed after the detector to eliminate further mobile phase out-gassing.

Place the mobile phase in the HPLC solvent reservoir and set the pump at a flowrate of 1,0 ml/min. Allow it to pump for 20 min to 30 min before the first analysis. Switch the detector on at least 30 min before the first analysis. Display the detector output on a strip chart recorder or similar output device.

For manual injection systems, draw at least 100 µl of the sample into a clean HPLC injection syringe. Fill the HPLC loop (load position of valve) by addition of excess sample via the syringe. Turn the valve to “inject” position to start the run. Activate the data system simultaneously with the injection, and mark the point of injection on the strip chart recorder. After approximately 1 min, return the injection valve to the load position and rinse or flush the syringe and valve with acetonitrile/water mixture in preparation for the next sample analysis.

Do not syringe solvent through the HPLC loop while the valve is in the “inject” position.

After elution of the DNPH-formaldehyde derivative (see Figure 4), terminate data acquisition and calculate the component concentrations as described in clause 10. After a stable baseline is achieved, the system can be used for further sample analysis as described above.

NOTE 1 After several cartridge analyses, buildup on the column (if indicated, e.g. by increasing pressure from run to run at a given flow and solvent composition) may be removed by flushing with several column volumes of 100 % acetonitrile. The same protection can be achieved if precolumns are used.

If the concentration of analyte exceeds the linear range of the instrument, the sample should be diluted with mobile phase, or a smaller volume injected into the HPLC. If the retention time found in earlier runs is not duplicated (± 10 %), the acetonitrile/water ratio may be increased or decreased to obtain the correct elution time. If the elution time is too long, increase the ratio; if it is too short, decrease the ratio. If a solvent change is necessary, always recalibrate (see 9.3.3) before running samples.

NOTE 2 The chromatographic conditions described here have been optimized for the detection of formaldehyde. Analysts are advised to experiment with their HPLC system to optimize chromatographic conditions for their particular analytical needs. HPLC systems with automated injection and start of data acquisition may also be used.

Examine the chromatogram for evidence of ozone interference according to 4.2 and Figure 2.

9.3.5 HPLC analysis of other aldehydes and ketones

9.3.5.1 General

Optimizing chromatographic conditions, by using two C18 columns in series and varying the mobile phase composition through a gradient programme, enables the analysis of other aldehydes and ketones collected from air. In particular, chromatographic conditions can be optimized to separate acetone, propionaldehyde and some higher molecular mass aldehydes within an analysis time of about 1 h.

The linear gradient programme varies the mobile phase composition periodically to achieve maximum resolution of the C_3, C_4 and benzaldehyde region of the chromatogram. The following gradient programme was found to be adequate to achieve this goal: upon sample injection, linear gradient in volume fraction from 60 % acetonitrile (ACN)/40 % water to 75 % ACN/25 % water in 36 min; to 100 % ACN in 20 min; 100 % ACN for 5 min; reverse linear gradient from 100 % ACN to 60 % ACN/40 % water in 1 min; maintain at 60 % ACN/ 40 % water for 15 min.

9.3.5.2 Sample analysis for other carbonyl compounds

Assemble and calibrate the HPLC system as described in 9.3.3 The operating parameters are as follows:

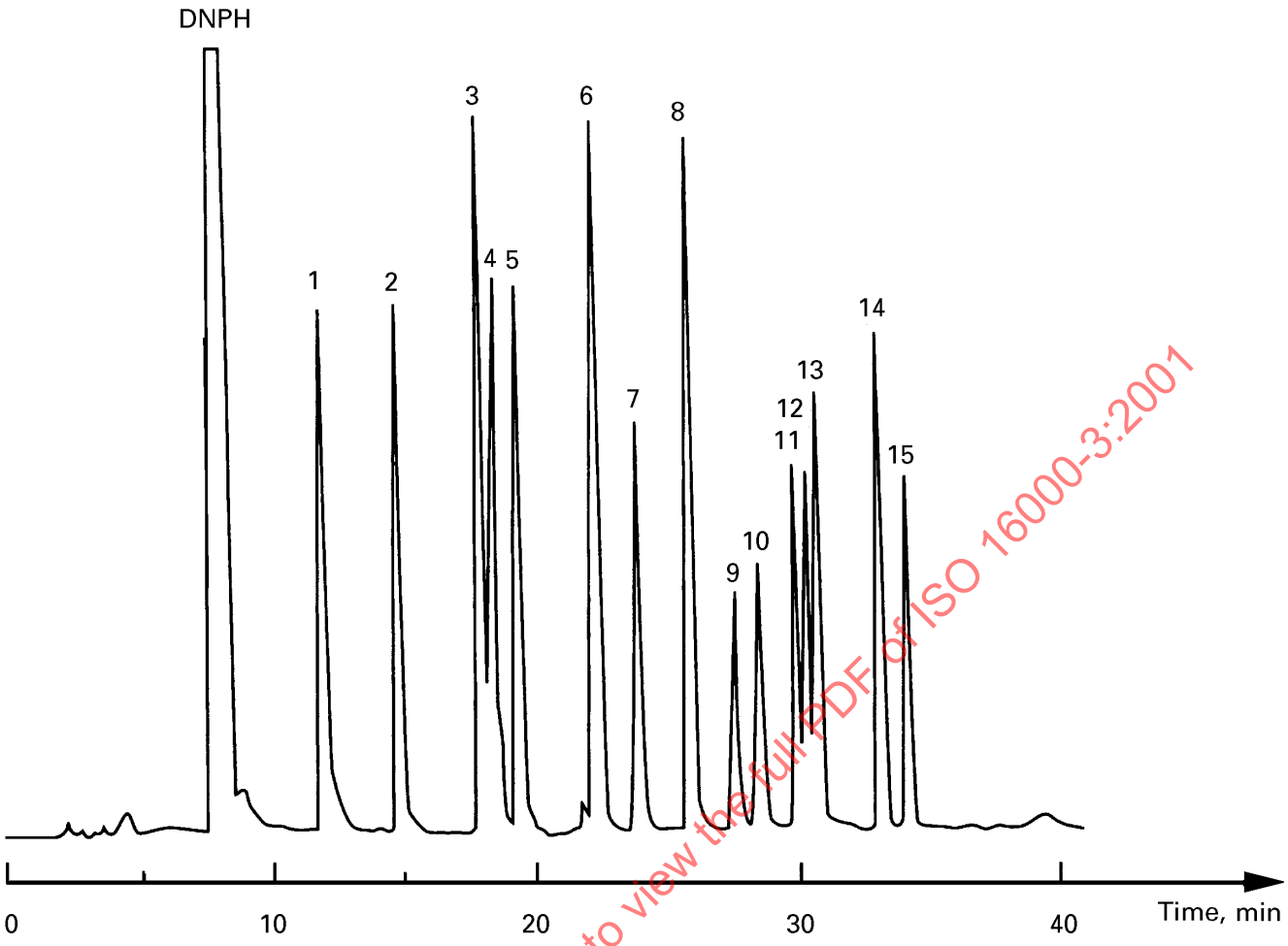
Column	C18, two columns in series
Mobile phase	Acetonitrile/water, linear gradient
Detector	Ultraviolet, operating at 360 nm
Flowrate	1,0 ml/min
Programme	See 9.3.4

The chromatographic conditions described herein have been optimized for a gradient HPLC system equipped with a UV detector or a diode array detector, an automatic sampler with a 25-µl loop injector and two C18 columns (4,6 mm × 250 mm), and a recorder or electronic integrator. Analysts are advised to experiment with their HPLC systems to optimize chromatographic conditions for their particular analytical needs. The separation of acrolein, acetone and propionaldehyde should be a minimum goal of the optimization.

NOTE Column manufacturers usually recommend optimal conditions for the separation of DNPH derivatives with their reverse phase columns. These recommendations may eliminate the need for dual columns without compromising resolution of the carbonyl compounds.

The carbonyl compounds in the sample are identified and quantified by comparing their retention times and number of area counts with those of standard DNPH derivatives. Formaldehyde, acetaldehyde, acetone, propionaldehyde, crotonaldehyde, benzaldehyde and *o*-, *m*-, *p*-tolualdehydes can be identified with a high degree of confidence. The identification of butyraldehyde is less certain, because it coelutes with isobutyraldehyde and methyl ethyl ketone under the stated chromatographic conditions. Figure 7 illustrates a typical chromatogram obtained with the gradient HPLC system.

The concentrations of individual carbonyl compounds are determined as outlined in 9.3.4.



Peak identification		
Number	**Compound**	**Concentration** µg/ml
1	Formaldehyde	1,140
2	Acetaldehyde	1,000
3	Acrolein	1,000
4	Acetone	1,000
5	Propionaldehyde	1,000
6	Crotonaldehyde	1,000
7	Butyraldehyde	1,000
8	Benzaldehyde	1,000
9	Isovaleraldehyde	0,450
10	Valeraldehyde	0,450
11	*o*-Tolualdehyde	0,515
12	*m*-Tolualdehyde	0,505
13	*p*-Tolualdehyde	0,510
14	Hexanal	1,000
15	2,5-Dimethylbenzaldehyde	0,510

Figure 7 — Example of chromatographic separation of DNPH derivatives of 15 carbonyl standards

10 Calculations

Calculate the total mass of analyte (DNPH derivative) for each sample using the following equation:

$$m_d = m_s - m_b \quad (3)$$

where

m_d is the corrected mass, in micrograms, of DNPH derivative extracted from cartridge,

m_s is the uncorrected mass, in micrograms, on the sample cartridge

$$= A_s \cdot (c_{std}/A_{std}) \cdot V_s \cdot d_s, \quad (4)$$

m_b is the analyte mass, in micrograms, on the blank cartridge

$$= A_b \cdot (c_{std}/A_{std}) \cdot V_b \cdot d_b, \quad (5)$$

where

A_s is the number of area counts, eluate from sample cartridge,

A_b is the number of area counts, eluate from blank cartridge,

A_{std} is the number of area counts, standard,

c_{std} is the concentration, in micrograms per millilitre, of analyte in the daily calibration standard,

V_s is the total volume, in millilitres, of the sample cartridge eluate,

V_b is the total volume, in millilitres, of the blank cartridge eluate,

d_s is the dilution factor for the sample cartridge eluate

$= 1$ if sample was not rediluted

$= V_d/V_a$ if sample was rediluted to bring the detector response within linear range, where

V_d is the redilution volume, in millilitres,

V_a is the aliquot used for redilution, in millilitres, and

d_b is the dilution factor for the blank cartridge eluate $= 1{,}0$.

Calculate the concentration of the carbonyl compound in the original sample from the following equation:

$$c_A = m_d \cdot (M_c / M_{der}) \times 1000/V_m \quad (6)$$

where

c_A is the concentration, in nanograms per litre, of carbonyl compound in the original sample,

V_m is the total air sample volume, in litres, under indoor conditions, from 9.1,

M_c is the molecular mass of carbonyl compound (for formaldehyde = 30),

M_{der} is the molecular mass of the DNPH derivative (for formaldehyde = 210).

NOTE The use of parts per billion or parts per million is deprecated. However, for the convenience of certain users, the following information is provided.

To convert the carbonyl compound concentration c_A to parts per billion (10^{-9}) as a volume fraction, use the following equation:

$$c_A = c_{As}(\text{ng/l}) \times 24{,}4 / M_c \quad (7)$$

where

c_A is the concentration of carbonyl compound, in parts per billion (10^{-9}) by volume,

c_{As} is the concentration, in nanograms per litre, of carbonyl compound in the original sample, calculated using the air volume corrected to 25 °C and 101,3 kPa (V_s);

24,4 is the ideal gas volume, in nanolitres per nanomole, corrected to 25 °C.

The corrected air volume at 25 °C and 101,3 kPa is calculated from the following equation:

$$V_S = \{(V_m \cdot p_A)/101{,}3\} \times \{298/(273 + T_A)\} \quad (8)$$

where

V_S is the total sample volume, in litres, at 25 °C and 101,3 kPa,

p_A is the average indoor pressure, in kilopascals,

T_A is the average indoor temperature, in degrees Celsius.

If it is desired to obtain a concentration result in terms of parts per million (10^{-6}) at standard conditions (25 °C and 101,3 kPa) to compare with standards stated in these terms, the volume sampled should not be corrected for temperature and pressure.

11 Performance criteria and quality assurance

11.1 General

This clause summarizes required quality assurance measures and provides guidance concerning performance criteria that should be achieved within each laboratory.

The user shall adhere to the rules of ISO 9000-1:1994, ISO 9000-2:1997, ISO 17025 and EN 45001:1989.

11.2 Standard Operating Procedures (SOPs)

Users should generate SOPs describing the following activities in their laboratory: assembly, calibration, and operation of the sampling system, with the manufacturer and model of equipment used; preparation, purification, storage, and handling of sampling reagent and samples; assembly, calibration, and operation of the HPLC system, with make and model of equipment used; and all aspects of data recording and processing, including lists of computer hardware and software used.

The SOPs should provide specific stepwise instructions and should be readily available to and understood by the laboratory personnel conducting the work. The SOPs shall be consistent with this part of ISO 16000.

11.3 HPLC system performance

Calculate the HPLC system efficiency according to the following equation:

$$\eta = 5{,}54\left(t_r / w_{1/2}\right)^2 \quad (9)$$

where

η is the column efficiency (theoretical plates),

t_r is the retention time of analyte, in seconds, and

$w_{1/2}$ is the width of the component peak at half-height, in seconds.

A column efficiency of > 5 000 theoretical plates should be obtained.

Relative standard deviation of response for replicate HPLC injections should be ± 10 % or less, day to day, for analyte calibration standards at 1 µg/ml or greater levels. At the 0,5 µg/ml level and below, the precision of replicate analyses can vary up to 20 % for some carbonyl compounds. Precision of retention times should be better than ± 7 % on any given day.

11.4 Sample loss

Sample loss can occur when the capacity of the sorbent is exceeded, or when the flowrate exceeds the maximum compatible with complete collection. This possibility can be guarded against by setting two sampling cartridges in series, and analysing the contents of each, or by analysing both sections of a two-section sorbent cartridge. Should the quantity of collected analyte in the back-up section exceed 15 % of the analyte collected by the primary sampling section, breakthrough can be assumed to have occurred and may have compromised the accuracy of the results.

12 Precision and uncertainty

As is the case for other compounds as well, the precision and uncertainty of the determination of formaldehyde in indoor air is influenced by the two parameters, the reproducibility of the analytical procedure and the variation over time of the analyte concentration in the air. It is reasonable to assume that, generally, the latter has a much higher effect than the former, although it is difficult to quantify the effect in view of the variability of source strengths and ventilation conditions.

Annex A gives some information on the magnitude of the errors relating to the analytical procedure.