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Textiles — Determination of formaldehyde —

Part 3: Free and hydrolysed formaldehyde (extraction method) by liquid chromatography

Textiles — Dosage du formaldéhyde —

Partie 3: Formaldéhyde libre et hydrolysé (méthode par extraction) par chromatographie liquide

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 38, *Textiles*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 248, *Textiles and textile products,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 14184 series can be found on the ISO website.

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Textiles — Determination of formaldehyde —

Part 3: Free and hydrolysed formaldehyde (extraction method) by liquid chromatography

WARNING — The use of this document can involve hazardous materials, operations and equipment. It does not purport to address all of the safety or environmental problems associated with its use. It refers only to technical suitability. It is the responsibility of the user to determine any legal obligations relating to health and safety, at any stage, prior to use. It has been assumed in the drafting of this document that the execution of its provisions is entrusted to appropriately qualified and experienced people.

1 Scope

This document specifies a method for determining the amount of free formaldehyde and formaldehyde extracted partly through hydrolysis by means of an extraction method. The method can be applied for the testing of textile fibres, fabrics or yarns.

NOTE This method, based on liquid chromatography (LC), is selective and not sensitive to coloured extracts and is intended to be used for precise quantification of formaldehyde.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Conformity

Compared with ISO 14184-1, the two analytical methods should give similar trends but not necessarily the same absolute result. Therefore, in cases of dispute, the method in this document shall be used in preference to ISO 14184-1 (see Note in Clause 1).

5 Principle

The sample is extracted with extraction solution at 40 °C. The eluate is mixed with 2,4-dinitrophenylhydrazine (DNPH), whereby formaldehyde reacts to give the respective hydrazone. It is separated by liquid chromatography with ultraviolet detector (LC-UV) or liquid chromatography with

diode array detector (LC-DAD) or liquid chromotagraphy with single quadrupole mass detector (LC-MS) or liquid chromatography with triple quadrupole mass detector (LC-MSMS) and the amount is quantified.

The process is selective. Formaldehyde is separated and quantified as a derivative from other aldehydes and ketones by LC. Free formaldehyde and formaldehyde which is hydrolysed during extraction to yield free formaldehyde is quantified.

6 Reagents

All reagents shall be of analytical reagent grade, unless otherwise stated.

6.1 Grade 3 water, in accordance with ISO 3696.

6.2 Acetonitrile (CAS Registry Number[1)] 75-05-8), LC -MS grade.

6.3 Formaldehyde solution CH_2O (CAS Registry Number[1)] 50-00-0), approximately 37 % (mass fraction).

6.4 Formaldehyde-2,4-DNPH certified reference material (CRM47177)[2)], 100 µg/ml.

Certified solutions of formaldehyde-2,4-DNPH, which are commercially available should be used. When these solutions are used, the procedure in 9.1 is not required.

6.5 Dinitrophenylhydrazine (DNPH) (CAS Registry Number[1)] 119-26-6) solution consisting of 0,3 g DNPH (2,4 dinitrophenylhydrazine) dissolved in 100 ml acetonitrile (6.2). DNPH commercially available reagent should be ≥ 97 % purity.

6.6 Iodine solution (CAS Registry Number[1)] 7553-56-2), 0,05 mol/l.

6.7 Sodium hydroxide solution (CAS Registry Number[1)] 1310-73-2), 2,0 mol/l.

6.8 Sulfuric acid solution (CAS Registry Number[1)] 7664-93-9), 2,0 mol/l.

6.9 Sodium thiosulfate solution (CAS Registry Number[1)] 10102-17-7), 0,1 mol/l.

6.10 Starch solution (CAS Registry Number[1)] 9005-84-9), 1 %., for example, 1 g in 100 ml water (6.1).

6.11 Sodium acetate (CAS Registry Number[1)] 127-09-3) ≥ 97 % purity.

6.12 Acetic acid (CAS Registry Number[1)] 64-19-7) ≥ 97 % purity.

6.13 Extraction solution. Acetic acid/sodium acetate buffer solution 0,1 mol/l (pH = 5,0).

Prepare 800 ml of distilled water (6.1) in a 1 000 ml flask. Add 9,53 g of Sodium acetate (6.11) and 2,7 g of Acetic acid (6.12). If necessary, adjust the pH with HCl or NaOH, then make up to volume with distilled water (6.1).

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1) Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

2) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

7 Apparatus

The usual laboratory apparatus and laboratory glassware shall be used, and in particular the following:

7.1 Stoppered volumetric flasks, for example,10 ml, 25 ml, 500 ml and 1 000 ml.

7.2 Conical flasks with stopper or screw cap, 250 ml.

7.3 Micro pipettes, for example, 10 µl to 100 µl, 100 µl to 1 000 µl and 1 ml to 5 ml.

7.4 Burettes, for example, 10 ml and 50 ml.

7.5 Water bath, thermostatically controlled to (40 ± 2) °C, fitted with a flask shaker, frequency (50 ± 10) $r \cdot min^{-1}$.

7.6 Water bath or oven, thermostatically controlled to (50 ± 2) °C.

7.7 Strainer with glass fibre filter, GF8 (or glass filter strainer G3, diameter 70 mm to 100 mm)**.**

7.8 Analytical balance, with the resolution of 0,1 mg.

7.9 LC system with UV, DAD, MS or MSMS detection.

7.10 Membrane filter, for example polyamide, 0,45 µm.

8 Preparation of test specimen

Do not condition the sample because the pre-drying and humidity in connection with the conditioning may cause changes in the formaldehyde content of the sample. Prior to testing, store the sample in a container.

Storage can be in a polyethylene bag and wrapped in aluminium foil. The reason for the storage precaution is that formaldehyde might diffuse through the pores of the bag. In addition, catalysts, or other compounds present in a finished, unwashed fabric, can react with the foil if in direct contact.

Cut the textile sample into pieces of about 0,3 cm to 0,5 cm edge length.

9 Procedure

9.1 Formaldehyde stock solution

9.1.1 Preparation of formaldehyde stock solution

The use of commercially available Certified Reference Material solutions (6.4) is recommended. In-house prepared stock solutions may be used only upon verification of precision data with the formaldehyde-2,4-DNPH certified reference material solution.

If in-house stock solution is used, pipette 5 ml of the formaldehyde solution (6.3) into a 1 000 ml volumetric flask (7.1) containing approximately 100 ml water (6.1) and fill the flask with water (6.1) up to the mark. This solution is the formaldehyde stock solution (S1).

9.1.2 Determination of the formaldehyde concentration in the stock solution

Pipette 5 ml from the solution prepared as in 9.1.1 into a 250 ml conical flask (7.2) and mix with 50 ml iodine solution (6.6). Add sodium hydroxide (6.7) until it turns yellow. Allow it to react for (15 ± 1) min at 18 °C to 26 °C and then add 15 ml of sulfuric acid (6.8) while swirling.

After adding 2 ml of starch solution (6.10), titrate the excess iodine with sodium thiosulfate (6.9) until the colour changes. Make three individual determinations.

Titrate at least two blank solutions in the same manner.

The concentration of formaldehyde stock solution is calculated according Formula (1):

$$\rho_{FA} = \frac{(V_0 - V_1) \times c_1 \times M_{FA}}{2} \quad (1)$$

where

ρ_{FA} is the concentration of the formaldehyde stock solution, in mg/10 ml;

V_0 is the titre of the thiosulfate solution for the blank solution, in ml;

V_1 is the titre of the thiosulfate solution for the sample solution, in ml;

M_{FA} is the relative molecular mass of formaldehyde, 30,02 g/mol;

c_1 is the concentration of the thiosulfate solution, in mol/l.

9.2 Determination of formaldehyde

9.2.1 Calibration of LC

9.2.1.1 General

Proposals for suitable LC conditions are given in Annex B.

9.2.1.2 Calibration with formaldehyde stock solution

At least four calibration solutions shall be used to cover the formaldehyde concentration range of 5 mg/kg to 100 mg/kg.

Prepare the standard solution (S2) for calibration purposes, i.e. the standard solution is approximately 4 µg/ml in formaldehyde content.

For example, pipette 1 ml of the formaldehyde stock solution (S1) obtained in 9.1.1, with a precisely know formaldehyde content, into a 500 ml volumetric flask (7.1), pre-filled with approximately 100 ml of extraction solution (6.13). Mix and fill to the mark with extraction solution (6.13), then mix again. Add the standard solution (S2) into each of four 25 ml volumetric flasks (7.1), for sample under the given conditions and fill the flasks (7.1) up to the mark with extraction solution (6.13) and mix.

Here is an example:

— 0,25 ml of S2 to 25 ml, containing 0,05 µg CH_2O/ml equivalent to 5 mg/kg CH_2O on the fabric

— 0,5 ml of S2 to 25 ml, containing 0,10 µg CH_2O/ml equivalent to 10 mg/kg CH_2O on the fabric

— 2,5 ml of S2 to 25 ml, containing 0,50 µg CH_2O/ml equivalent to 50 mg/kg CH_2O on the fabric

— 5,0 ml of S2 to 25 ml, containing 1,00 µg CH_2O/ml equivalent to 100 mg/kg CH_2O on the fabric

Pipette 5 ml of each formaldehyde calibration solution, into 10 ml volumetric flasks (7.1), pre-filled with 4 ml acetonitrile (6.2). Immediately upon addition of the calibration solution, mix each flask and add 0,5 ml DNPH solution (6.5). Fill the flasks up to the mark with extraction solution (6.13) and mix. Place the flasks in a water bath or oven (7.6) preheated at (50 ± 2) °C per (180 ± 2) min. Then analyse the calibration solutions using liquid chromatography (LC-UV or LC-DAD or LC-MS or LC-MSMS).

Calculate the first-order regression curve of the type y = a + bx. This regression curve will be used for all measurements. If the test specimen contains a higher amount of formaldehyde than 500 mg/kg, dilute the sample solution.

9.2.1.3 Calibration with derivatized DNPH-formaldehyde

At least, four calibration solutions shall be used. Add the formaldehyde-2,4-DNPH (6.4) into each of four 25 ml volumetric flasks (7.1) pre-filled with 4 ml acetonitrile (6.2), in order to cover the formaldehyde concentration range of 5 mg/kg to 100 mg/kg on sample, under the given conditions, and fill the flasks (7.1) up to the mark with demineralized water (6.1) and mix.

Here is an example:

— 0,01 ml of formaldehyde-2,4-DNPH (6.4) to 25 ml, containing 0,05 µg CH_2O/ml equivalent to 5 mg/kg CH_2O on the fabric

— 0,02 ml of formaldehyde-2,4-DNPH (6.4) to 25 ml, containing 0,10 µg CH_2O/ml equivalent to 10 mg/kg CH_2O on the fabric

— 0,1 ml of formaldehyde-2,4-DNPH (6.4) to 25 ml, containing 0,50 µg CH_2O/ml equivalent to 50 mg/kg CH_2O on the fabric

— 0,2 ml of formaldehyde-2,4-DNPH (6.4) to 25 ml, containing 1,00 µg CH_2O/ml equivalent to 100 mg/kg CH_2O on the fabric

Plot the concentrations in micrograms per ml in a calibration graph against the measured formaldehyde derivative peak area. X-axis: concentration in micrograms per ml, y-axis: peak area.

9.2.2 Extraction of the test specimen

For each test specimen, put (2,0 ± 0,1) g of test pieces (see Clause 8) into a 250 ml flask with a stopper (7.2) and record the mass to the nearest of 10 mg. Add 100 ml of extraction solution (6.13). Stopper tightly and place in a water bath (7.5) at (40 ± 2) °C for (60 ± 5) min. Shake the flask at least every 5 min, ensuring that the test specimens are entirely wet. Then filter the solution into another flask through a filter (7.7).

If it is difficult for the test specimens to be completely wet, a mechanical-shaking water bath should be used.

Immediately after the extraction of the test specimen, proceed with the reaction with DNPH as reported below.

9.2.3 Derivatization with DNPH and analysis

9.2.3.1 Pipette 4,0 ml of acetonitrile (6.2), 5,0 ml aliquot of the filtered eluate (9.2.1) and 0,5 ml of DNPH solution (6.5) into a 10 ml volumetric flask (7.1). Place the flask in the water bath or oven (7.6) preheated at (50 ± 2) °C for (180 ± 2) min. Cool the flask down to room temperature (18 °C to 26 °C). Fill the volumetric flask with extraction solution (6.13) up to the mark and shake it briefly by hand to mix the components. If necessary, filter through a membrane filter (7.10) and then analyse the solution with liquid chromatography (7.9) (LC-UV or LC-DAD or LC-MS or LC-MSMS).

Examples of chromatographic and spectroscopic conditions are given in Annex B.

9.2.3.2 For a high content of formaldehyde (> 500 mg/kg), make aliquots smaller than 5 ml up to 5 ml with extraction solution (6.13). Example of the procedure when formaldehyde content is approximately 500 mg/kg: pipette 4,0 ml of acetonitrile (6.2), a 0,5 ml aliquot of the filtered eluate (9.2.1), 4,5 ml of extraction solution (6.13) and 0,5 ml of DNPH solution (6.5) into a 10 ml volumetric flask (7.1). Then follow the procedure as described in 9.2.3.1.

10 Expression of results

10.1 Calculation of the formaldehyde content in textile test specimen

The concentration of formaldehyde in the textile test specimen is calculated according to Formula (2):

$$w_F = \frac{\rho_S \times F}{m} \quad (2)$$

where

w_F is the mass fraction of formaldehyde in the textile test specimen in mg/kg rounded to 0,1 mg/kg;

ρ_S is the mass concentration of formaldehyde obtained from the calibration graph in µg/ml;

F is the dilution factor in ml, usually 200 ml (100 ml extraction volume × 2 for an aliquot);

m is the mass of weighed textile test specimen in g.

10.2 Spiking — Determination of recovery rate

Determination of the recovery rate is not mandatory. If necessary, the following procedure can be used.

Put 4 ml acetonitrile (6.2) into a 10 ml volumetric flask (7.1) and add an aliquot of 2,5 ml of the filtrate, obtained as described in 9.2.1. Then add an adequate volume of the formaldehyde standard solution.

Further treat this solution following the procedure described in 9.2.2 and determine ρ_{S2} following the same procedure. Carry out the determination and report the value in the test report.

The recovery rate is calculated according to Formula (3).

$$R_R = \frac{(\rho_{S2} - 0{,}5\rho_{S1}) \times 100}{\rho_{FA1}} \quad (3)$$

where

R_R is the recovery rate in per cent, rounded off to 0,1 %;

ρ_{S2} is the concentration of formaldehyde obtained from the calibration graph in µg/ml;

ρ_{S1} is the concentration of formaldehyde in the non-spiked sample in µg/ml;

ρ_{FA1} is the spiked quantity of formaldehyde in µg/ml.

10.3 Precision of the test method

The procedure is suggested for use in the working range of free and hydrolysed formaldehyde on the fabric between 5 mg/kg and 100 mg/kg. The limit of quantification should be not lower than 5 mg/kg. Below this limit, the result should be reported as “below LOQ” (Limit Of Quantification)or “below 5 mg/kg”.

For the reliability (precision) of the procedure, see Annex A.

11 Test report

The test report shall include the following information:

a) description of the sample;

b) a reference to this document, i.e. ISO 14184-3:2023;

c) the calibration method;

d) the mass fraction of formaldehyde in mg/kg (10.1);

e) any deviations from the procedure;

f) any unusual features observed;

g) the date of the test.

Annex A
(informative)

Information on precision of the test method

In 2022, a collaborative trial for the validation of this method for the determination of formaldehyde has been organized including 17 laboratories. The interlaboratory study concerned 4 different samples of textile, 3 textile fortified samples: one sample around 20 ppm (part per million) one sample around 80 ppm (part per million) one sample around 120 ppm (part per million), additionally has been sent a blank sample. Approximately 10 grams per sample has been sent to each lab. It has been required to treat the samples as if they were routine samples and analyse them in day-to-day circumstances. It has been required to follow both ISO 14184-3 and ISO 14184-1 in order to compare results obtained with different techniques. To ensure the homogeneity it has been required to do not use less than 2,0 gram per determination, each laboratory has been required to test each sample in double. The analytical techniques that can be used for the determination of the formaldehyde content were as follows for ISO 14184-3.

— LC-DAD

— LC-MS

— LC-MSMS

NOTE Each laboratory used the ones available at its own sites.

Results for LC-MS and LC-MSMS were collected together.

See Tables A.1, A.2, A.3.

Table A.1 — Formaldehyde content determined using LC-MS or LC-MSMS

Textile sample	Mean formaldehyde content mg/kg	S_R	S_r
1	32,7	7,4	2,2
2	96,4	11,2	3,5
3	134,2	26,8	15,5

The data in Table A.1 were obtained in an interlaboratory trial on textile samples with unknown levels of formaldehyde. Eight of the 17 laboratories sent results for this technique. Applying ISO 5725-2, the statistical outliers identified with Cochran's tests were rejected. Only one laboratory has been identified as outlier for second and third textile samples.

Table A.2 — Formaldehyde content determined using LC-DAD

Textile sample	Mean formaldehyde content mg/kg	S_R	S_r
1	31,2	5,0	0,7
2	91,2	11,1	4,0
3	124,1	14,5	5,1

The data in Table A.2 were obtained in an interlaboratory trial on textile samples with unknown levels of formaldehyde. Twelve of the above mentioned 17 laboratories sent results for this technique. Applying ISO 5725-2, the statistical outliers identified with Cochran's tests were rejected. Only one laboratory has been identified as outlier for second and third textile samples.

Table A.3 — Formaldehyde content determined using UV following ISO 14184-1

Textile sample	Mean Formaldehyde content mg/kg	S_R	S_r
1	31,3	5,8	2,2
2	86,4	9,4	2,0
3	115,5	10,9	3,4

The data in Table A.3 were obtained in an interlaboratory trial on textile samples with unknown levels of formaldehyde. Thirteen of the above mentioned 17 laboratories sent results for this technique. Applying ISO 5725-2, the statistical outliers identified with Cochran's tests were rejected. Only one laboratory has been identified as outlier for second and third textile samples.

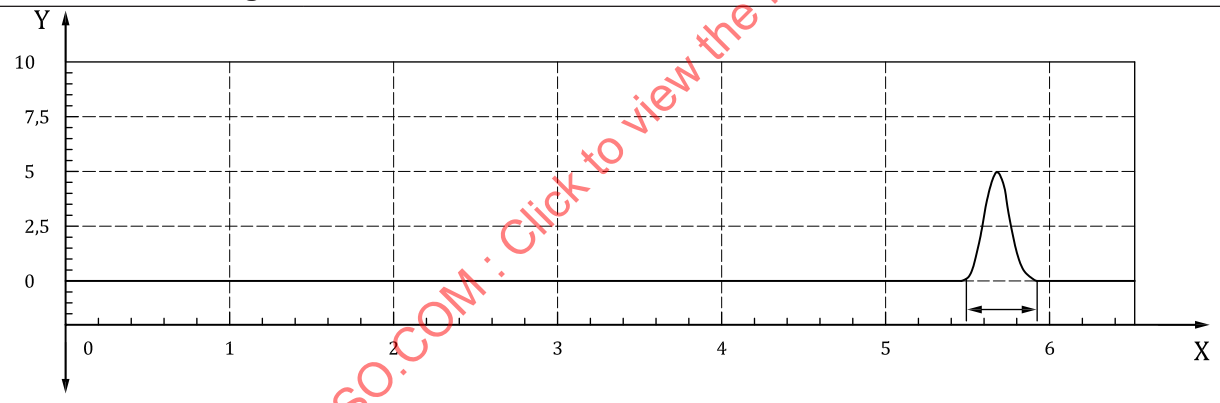
Annex B
(informative)

Examples of chromatographic and spectroscopic conditions

B.1 Determination by LC-DAD

Figure B.1 shows an example of LC-DAD chromatogram.

LC	
Column flow	1 ml/min
Solvent program	Isocratic (acetonitrile/water, 60: 40)
Separation column	Agilent HC-C18 reversed phase column (150 mm × 4,6 mm × 5 µm)
Injection volume	20 µl
Run time	7 min
DAD	
UV detection wavelength	360 nm



Key
X time (min)
Y absorbance

Figure B.1 — Example of LC-DAD Chromatogram — Derivatized DNPH-formaldehyde standard solution 10 mg/10 ml

B.2 Determination by LC-MS

Figure B.2 shows an example of LC-MS chromatogram.

LC	
Column flow	0,3 ml/min
Solvent program	Isocratic (10 mM ammonium acetate pH 3,6 / acetonitrile, 58: 42)
Separation column	Zorbax Eclipse XDB-C18 reversed phase column (150 mm × 2,1 mm × 5 µm)
Injection volume	1 µl