INTERNATIONAL STANDARD

ISO 17226-1

IULTCS/IUC 19-1

Second edition
2018-12

Leather — Chemical determination of formaldehyde content —

Part 1: Method using high performance liquid chromatography

Cuir — Dosage chimique du formaldéhyde —

Partie 1: Méthode par chromatographie en phase liquide à haute performance

Reference numbers
ISO 17226-1:2018(E)
IULTCS/IUC 19-1:2018(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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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.

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 documents 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 289, *Leather*, in collaboration with the Chemical Test Commission of the International Union of Leather Technologists and Chemists Societies (IUC Commission, IULTCS), in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). This method is technically similar to the Colorimetric Section of the method IUC 19 which was declared an official method at the IULTCS Delegates meeting on 31st May 2003 in Cancún, Mexico.

IULTCS, originally formed in 1897, is a world-wide organization of professional leather societies to further the advancement of leather science and technology. IULTCS has three Commissions, which are responsible for establishing international methods for the sampling and testing of leather. ISO recognizes IULTCS as an international standardizing body for the preparation of test methods for leather.

This second edition cancels and replaces the first edition (ISO 17226-1:2008), which has been technically revised as follows:

— the former Clause 2 has become Clause 4, a new Clause 3, *Terms and definitions*, inserted and subsequent clauses renumbered;

— 6.1.1, 6.2.1, 7.4, 7.6, 8.2.2, 8.2.3, 8.2.4.1, 8.2.4.2 and 8.2.6 have been technically modified;

— the recommended HPLC conditions, previously in 7.2.4, have been moved to an informative Annex B.

A list of all parts in the ISO 17226 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Leather — Chemical determination of formaldehyde content —

Part 1:
Method using high performance liquid chromatography

1 Scope

This document specifies a method for the determination of free and released formaldehyde in leathers. This method, based on high performance liquid chromatography (HPLC), is selective and not sensitive to coloured extracts and is intended to be used for precise quantification of formaldehyde.

The formaldehyde content is taken to be the quantity of free-formaldehyde and formaldehyde extracted through hydrolysis contained in a water extract from the leather under standard conditions.

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 2418, *Leather — Chemical, physical and mechanical and fastness tests — Sampling location*

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

ISO 4044, *Leather — Chemical tests — Preparation of chemical test samples*

ISO 4684, *Leather — Chemical tests — Determination of volatile matter*

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

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

4 Conformance

When compared with ISO 17226-2, 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 17226-2. See Annex A for additional information.

5 Principle

The process is selective. Formaldehyde is separated and quantified as a derivative from other aldehydes and ketones by liquid chromatography. Detected is the free-formaldehyde and formaldehyde which is hydrolysed during extraction to yield free-formaldehyde.

The sample is eluted with a detergent solution at 40 °C. The eluate is mixed with 2,4-dinitrophenylhydrazine, whereby aldehydes and ketones react to give the respective hydrazones. These are separated by means of a reversed-phase HPLC method, detected at (355 ± 5) nm and quantified.

6 Reagents

Use only reagents of recognized analytical grade, unless otherwise stated. The water shall be grade 3 in accordance with ISO 3696. All solutions are aqueous solutions.

6.1 Reagents for the formaldehyde stock solution

6.1.1 Formaldehyde solution, approximately 37 % (mass fraction).

Certified solutions of formaldehyde or formaldehyde-2,4-DNPH are commercially available. When these solutions are used, the procedure in 8.1.2 is not required.

6.1.2 Iodine solution, 0,05 mol/l, i.e. 12,68 g iodine per litre.

6.1.3 Sodium hydroxide solution, 2,0 mol/l.

6.1.4 Sulfuric acid solution, 2,0 mol/l.

6.1.5 Sodium thiosulfate solution, 0,1 mol/l.

6.1.6 Starch solution, 1 %, i.e. 1 g in 100 ml water.

6.2 Reagents for the HPLC method

6.2.1 Sodium dodecylsulfonate or sodium dodecylsulfate (detergent solution), 0,1 %, 1 g in 1 000 ml water.

6.2.2 Dinitrophenylhydrazine (DNPH) solution, consisting of 0,3 g DNPH (2,4-dinitrophenylhydrazine) dissolved in 100 ml concentrated *o*-phosphoric acid (85 % mass fraction). (DNPH recrystallized from 25 % mass fraction, acetonitrile in water.)

6.2.3 Acetonitrile HPLC grade.

7 Apparatus

Use the usual laboratory equipment and, in particular, the following:

7.1 Volumetric flasks, of capacities 10 ml, 500 ml and 1 000 ml.

7.2 Erlenmeyer flasks, of capacities 100 ml and 250 ml.

7.3 Strainer with glass fibre filter, GF8 (or glass filter strainer G 3, diameter 70 mm to 100 mm).

7.4 Water bath, thermostatically controlled to (40 ± 1) °C, fitted with a flask shaker, frequency (50 ± 10) min^{-1}.

7.5 Thermometer, with 0,1 °C graduations over the range 10 °C to 50 °C.

7.6 HPLC system with UV detection, (355 ± 5) nm or other validated apparatus.

7.7 Membrane filter, polyamide, 0,45 µm.

7.8 Analytical balance, weighing to an accuracy of 0,1 mg.

8 Procedures

8.1 Procedure for the determination of formaldehyde in the stock solution

8.1.1 Preparation of the formaldehyde stock solution

Pipette 5 ml of the formaldehyde solution (6.1.1) into a 1 000 ml volumetric flask (7.1) containing approximately 100 ml water and then fill the flask with demineralized water up to the mark. This solution is the formaldehyde stock solution.

8.1.2 Determination

Pipette 10 ml from this solution into a 250 ml Erlenmeyer flask (7.2) and mix with the 50 ml iodine solution (6.1.2). Add sodium hydroxide (6.1.3) 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 (6.1.4) while swirling.

After adding 2 ml of starch solution (6.1.6), titrate the excess iodine with sodium thiosulfate (6.1.5) until the colour changes. Make three individual determinations. Titrate at least two blank solutions in the same manner.

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

where

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

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

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

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

c_1 is the concentration of the thiosulfate solution, in moles per litre (mol/l).

8.2 Procedure for the determination of formaldehyde in leather by the HPLC method

8.2.1 Sampling and preparation of samples

Sample in accordance with ISO 2418. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments), provide details about sampling together with the test report. Cut leather in accordance with ISO 4044.

If the result is to be presented on the basis of dry substance, then test a further sample of the same leather in accordance with ISO 4684 so that the moisture content can be calculated.

8.2.2 Extraction

Weigh approximately (2 ± 0,1) g of leather pieces to the nearest 0,01 g into a 100 ml glass Erlenmeyer flask.

Add 50 ml of detergent solution (6.2.1) (previously preheated at 40 °C) and fit the Erlenmeyer flask with a glass stopper. Shake the contents of the flask in the water bath (7.4) for (60 ± 2) min at (40 ± 1) °C. Immediately filter the warm extract solution by vacuum (use not less than 50 mbar) through a glass fibre filter (7.3) into a flask. Cool the filtrate, in a closed flask, down to room temperature (18 °C to 26 °C).

Do not modify the leather/solution ratio. The extraction and analysis should be performed within the same working day.

8.2.3 Reaction with DNPH

Pipette 4,0 ml of acetonitrile (6.2.3), a 5,0 ml aliquot of the filtered eluate (8.2.2) and 0,5 ml of DNPH solution (6.2.2) into a 10 ml volumetric flask (7.1). Fill the volumetric flask with demineralized water up to the mark and shake it briefly by hand to mix the components. Allow it to stand for at least 60 min, but not more than a maximum of 180 min. After filtering through a membrane filter (7.7), analyse the sample using HPLC.

For a high content of formaldehyde (>100 mg/kg), make aliquots smaller than 5 ml up to 5 ml with water. Example of the procedure when formaldehyde content is approximately 500 mg/kg: pipette 4,0 ml of acetonitrile (6.2.3), a 0,5 ml aliquot of the filtered eluate (8.2.2), add 4,5 ml water and 0,5 ml of DNPH solution (6.2.2) into a 10 ml volumetric flask (6.1). Then follow the procedure as described in the previous paragraph.

See Annex B for additional information.

8.2.4 Calibration of HPLC

8.2.4.1 Calibration with formaldehyde stock solution

Pipette 1 ml of the formaldehyde stock solution obtained in 8.1.1, with a precisely known formaldehyde content, into a 500 ml volumetric flask (7.1), pre-filled with approximately 100 ml water. Mix together and fill to the mark with water, then mix again. This solution is the standard solution for calibration purposes, i.e. the standard solution is approximately 2 µg formaldehyde/ml.

In each of six 10-ml volumetric flasks (7.1), insert 4 ml acetonitrile (6.2.3), then add a concentration series of 0,25 ml; 1,0 ml; 2,0 ml; 3,0 ml; 4,0 ml; and 5,0 ml, respectively, of the standard solution. These solutions cover the formaldehyde concentration range of 0,1 µg/ml to 2,0 µg/ml. (This corresponds to a formaldehyde in leather concentration range of 5 mg/kg to 100 mg/kg leather under the given conditions.)

Immediately upon addition of the formaldehyde solution (6.1.1), mix each flask and add 0,5 ml DNPH solution (6.2.2). Fill the flasks up to the mark with demineralized water and mix. After at least 60 min and not more than 180 min, analyse the samples using HPLC after filtration through a membrane filter (7.7).

Plot the concentrations in micrograms per 10 ml in a calibration graph against the measured formaldehyde derivative peak area. *x*-axis: concentration in micrograms per 10 ml, *y*-axis: peak area.

8.2.4.2 Calibration with derivatised DNPH-formaldehyde

In each of six 10-ml volumetric flasks (7.1), insert 4 ml acetonitrile (6.2.3), then add an adequate quantity of derivatized DNPH-formaldehyde to ensure you have 1,0 µg; 4,0 µg; 8,0 µg; 12,0 µg; 16,0 µg; and 20,0 µg, respectively, of formaldehyde.

Fill the flasks up to the mark with demineralized water and mix. After at least 60 min and not more than 180 min, analyse the samples using HPLC after filtration through a membrane filter (7.7). For the calibration plot a graph of the formaldehyde derivative peak area versus the concentration in micrograms per 10 ml.

8.2.5 Calculation of the formaldehyde content in leather samples

$$w_F = \frac{\rho_S \times F}{m}$$

where

w_F is the concentration of formaldehyde in the sample in milligrams per kilogram (mg/kg) rounded to 0,01 mg/kg;

ρ_S is the concentration of formaldehyde obtained from the calibration graph in micrograms per 10 ml (µg/10 ml);

F is the dilution factor in millilitres (ml);

m is the mass of leather weighed in grams (g).

8.2.6 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 (6.2.3) into a 10 ml volumetric flask (7.1) and add an aliquot of 2,5 ml of the filtrate, obtained as described in 8.2.2. Then add an accurately determined volume of the formaldehyde standard solution to give an almost equal concentration to that found in the sample.

Further treat this solution following the procedure described in 8.2.3 and determine ρ_{S2} following the same procedure. Carry out the determination and report the value in the test report.

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

where

ρ_{S2} is the concentration of formaldehyde obtained from the calibration graph in micrograms per 10 ml (µg/10 ml);

ρ_S is the concentration of formaldehyde in the non-spiked sample in micrograms per 10 ml (µg/10 ml);

ρ_{FA1} is the spiked quantity of formaldehyde in micrograms per 10 ml (µg/10 ml);

R_R is the recovery rate in percent, rounded off to 0,1 %.

9 Expression of results

Express the formaldehyde concentration to the nearest 0,1 mg/kg based on the mass of the leather sample tested.

If the results are to be reported on the basis of dry substance, multiply the results above by the factor 100/(100 − w), where w is the moisture content in percent (%) according to ISO 4684. If the results are presented on the basis of dry substance, clearly mention this in the test report.

10 Test report

The test report shall include the following:

a) reference to this document (i.e. ISO 17226-1);

b) type, origin and designation of the analysed leather sample and the sampling method used;

c) the analytical procedure used;

d) the analytical results for the formaldehyde content;

e) any deviations from the analytical procedure, particularly any additional steps performed;

f) the date of the test;

g) if the results are determined on the basis of the dry substance this shall be reported.

Annex A
(informative)

Precision: reliability of the chromatographic HPLC method

The following data were obtained in a collaborative trial with 10 laboratories on leather samples with unknown levels of formaldehyde.

Table A.1 — Reliability data of the chromatographic HPLC method

Leather sample	Mean formaldehyde content mg/kg	Repeatability *r* mg/kg	Reproducibility *R* mg/kg	Recovery rate %
A	7,65	1,27	3,13	94
B	17,69	3,82	7,97	96
C	28,69	5,40	11,42	91
D	102,16	20,82	64,33	94