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17226-1**

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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

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 17226-1 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 sampling and the testing of leather. ISO recognizes IULTCS as an international standardizing body for the preparation of test methods for leather.

This first edition of ISO 17226-1, together with ISO 17226-2, cancels and replaces ISO/TS 17226:2003, which has been technically revised.

ISO 17226 consists of the following parts, under the general title *Leather — Chemical determination of formaldehyde content*:

— *Part 1: Method using high performance liquid chromatography*

— *Part 2: Method using colorimetric analysis*

Leather — Chemical determination of formaldehyde content —

Part 1:
Method using high performance liquid chromatography

1 Scope

This part of ISO 17226 specifies a method for the determination of free and released formaldehyde in leathers. This method is based on high performance liquid chromatography (HPLC). It is selective and not sensitive to coloured extracts.

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 Conformance

When compared with ISO 17226-2, the two analytical methods should give similar trends but not necessarily the same absolute result. Therefore, in the case of dispute, this method (ISO 17226-1) shall be used in preference to ISO 17226-2.

3 Normative references

The following referenced documents are indispensable for the application 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 3696:1987, *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*

4 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 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 360 nm and quantified.

5   Reagents

Use only reagents of recognized analytical grade, unless otherwise stated. The water shall be grade 3 in accordance with ISO 3696:1987. All solutions are aqueous solutions.

5.1   Reagents for the formaldehyde stock solution

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

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

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

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

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

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

5.2   Reagents for the HPLC method

5.2.1   Sodium dodecylsulfonate (detergent) solution, 0,1 %, 1 g in 1 000 ml water.

5.2.2   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)

5.2.3   Acetonitrile.

6   Apparatus

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

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

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

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

6.4   Water bath, thermostatically controlled to 40 °C $\pm$ 0,5 °C, fitted with a flask shaker or stirrer.

6.5   Thermometer, with 0,1 °C graduations over the range 20 °C to 50 °C.

6.6   HPLC system with UV detection, 360 nm.

6.7   Membrane filter, polyamide, 0,45 μm.

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

7 Procedures

7.1 Procedure for the determination of formaldehyde in the stock solution

7.1.1 Preparation of the formaldehyde stock solution

Pipette 5 ml of the formaldehyde solution (5.1.1) into a 1 000 ml volumetric flask (6.1) which contains approximately 100 ml water and then fill the flask with demineralized water up to the mark. This solution is the formaldehyde stock solution.

7.1.2 Determination

Pipette 10 ml from this solution into a 250 ml Erlenmeyer flask (6.2) and mix with the 50 ml iodine solution (5.1.2). Add sodium hydroxide (5.1.3) until it turns yellow. Allow it to react for 15 min $\pm$ 1 min at 18 °C to 26 °C and then add 15 ml of sulfuric acid (5.1.4) while swirling.

After adding 2 ml of starch solution (5.1.6), titrate the excess iodine with sodium thiosulfate (5.1.5) 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).

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

7.2.1 Sampling and preparation of samples

If possible, 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. Grind the 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.

7.2.2 Extraction

Weigh 2 g $\pm$ 0,1 g leather into a suitable vessel. Pipette 50 ml of the detergent solution (5.2.1) into a 100 ml Erlenmeyer flask (6.2) and warm it to 40 °C. Transfer the pre-weighed leather quantitatively to the flask, then close it with a glass stopper (see next paragraph). Stir the content of the flask or shake it smoothly at 40 °C $\pm$ 0,5 °C in a water bath (6.4) for 60 min $\pm$ 2 min. Immediately filter the warm extract solution by vacuum through a glass fibre filter (6.3) 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. Extraction and analysis should be performed within the same working day.

7.2.3 Reaction with DNPH

Pipette 4,0 ml of acetonitrile (5.2.3), a 5,0 ml aliquot of the filtered eluate (7.2.2) and 0,5 ml of DNPH solution (5.2.2) into a 10 ml volumetric flask (6.1). 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 at least 60 min, but not more than a maximum of 180 min. After filtering through a membrane filter (6.7), analyse the sample using HPLC. If the concentration is out of the calibration range, take smaller aliquots.

7.2.4 HPLC conditions (recommendations)

These conditions are only recommendations. The method used should be verified using the recovery rate determination (7.2.7) and the results observed should be in the range listed in Table A.1.

Flow rate:	1,0 ml/min
Mobile phase:	acetonitrile/water, 60:40
Separation column:	C18 reversed phase column with precolumn (1 cm, RP18)
UV detection wavelength:	360 nm
Injection volume:	20 µl

NOTE   A Merck 100, CH 18,2 (highly coated, 12 % C) column is an example of a suitable separation column which is commercially available.[1]

7.2.5 Calibration of HPLC

Pipette 0,5 ml of the formaldehyde stock solution obtained in 7.1.1, with an exactly known formaldehyde content, into a 500 ml volumetric flask (6.1), pre-filled with approximately 100 ml water. Mix together and fill to the mark with water, and 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 (6.1), add 4 ml acetonitrile (5.2.3), then add a concentration series of 0,5 ml; 1,0 ml; 2,0 ml; 3,0 ml; 4,0 ml; 5,0 ml, respectively, of the standard solution. Immediately upon addition of the formaldehyde solution (5.1.1), mix each flask and add 0,5 ml DNPH solution (5.2.2). 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 (6.7). Effect the calibration through plotting a graph of the formaldehyde derivative peak area versus the concentration in micrograms per 10 ml.

7.2.6 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);

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1)   A Merck 100, CH 18,2 column is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

F is the dilution factor in millilitres (ml);

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

7.2.7 Spiking — Determination of recovery rate

Pre-fill 4 ml acetonitrile (5.2.3) into a 10 ml volumetric flask (6.1) and add an aliquot of 2,5 ml of the filtrate, obtained as described in 7.2.2. 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 7.2.3 and determine ρ_{S2} following the procedure described in 7.2.3. Carry out the determination and report the value in the test report.

$$R_{R} = \frac{\left(\rho_{S2} - 0,5\ \rho_{S}\right) \times 100}{\rho_{FA1}}$$

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 %.

8 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.

9 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.