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**ISO 17226-2**

**IULTCS/IUC 19-2**

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# Leather — Chemical determination of formaldehyde content —

## Part 2: Method using colorimetric analysis

*Cuir — Dosage chimique du formaldéhyde —*

*Partie 2: Méthode par analyse colorimétrique*

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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 sampling and the 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 17226-2:2008), which has been technically revised. It also incorporates the Technical Corrigendum ISO 17226-2:2008/Cor.1:2009. The main changes compared to the previous edition are as follows:

— Clause 1 has been modified;

— 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, 6.2.2, 7.4, 7.7, 8.2.2, 8.2.3, 8.2.4, 8.2.6 and 8.2.8 have been technically modified.

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.

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# Leather — Chemical determination of formaldehyde content —

# Part 2:
# Method using colorimetric analysis

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## 1 Scope

This document specifies a method for the determination of free and released formaldehyde in leathers. This method, based on colorimetric analysis, is not intended to be used for a 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.

This process is not absolutely selective for formaldehyde. Other compounds such as extracted dyes could interfere at 412 nm.

## 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*

ISO 17226-1, *Leather — Chemical determination of formaldehyde content — Part 1: Method using high performance liquid chromatography*

## 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 17226-1, the two analytical methods should give similar trends but not necessarily the same absolute result. Therefore, in cases of dispute, ISO 17226-1 shall be used in preference to this document.

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## 5 Principle

The leather sample is eluted with detergent solution at 40 °C. The eluate is treated with acetylacetone, whereby formaldehyde reacts to give a yellow compound (3,5-diacetyl-1,4-dihydrolutidine). The absorbance of this compound is measured at 412 nm. The amount of formaldehyde corresponding to the absorbance value for the test specimen is obtained from a calibration curve prepared under identical conditions.

## 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 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 colorimetric method

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

**6.2.2 Solution 1**, 150 g ammonium acetate + 3 ml glacial acetic acid + 2 ml acetylacetone (pentane-2,4-dione, CAS 123-54-6) in 1 000 ml water.

The solution can be stored for one week in a dark place (it is sensitive to light).

**6.2.3 Solution 2**, 150 g ammonium acetate + 3 ml glacial acetic acid in 1 000 ml water.

**6.2.4 Dimedone solution**, 5 g dimedone[1)] in 1 000 ml water. Prepare immediately before use.

In some cases, dimedone cannot be readily dissolved in pure water. In such cases, dimedone can be dissolved in a small amount of ethanol and then made up to volume with water.

## 7 Apparatus

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

**7.1 Volumetric flasks**, of capacities 10 ml, 50 ml and 1 000 ml.

1) Dimedone (CAS 126-81-8) or methone is 5,5'-dimethyl-1,3-cyclohexanedione.

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**7.2 Erlenmeyer flasks**, of capacities 25 ml, 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 Analytical balance**, weighing to an accuracy of 0,1 mg.

**7.7 Spectrophotometer**, with suitable semi-micro cells capable of measuring absorbance at 412 nm.

The recommended cell path length is 20 mm. To increase the sensibility, a semi-micro cell with 40 mm or 50 mm optical path length can be used.

# 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 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

$\rho_{FA}$ 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).

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## 8.2 Procedure for the determination of formaldehyde in leather by the colorimetric 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 an additional 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. Extraction and analysis should be performed within the same working day.

### 8.2.3 Reaction with acetylacetone

Pipette 5 ml of the filtrate obtained in 8.2.2 into a 25 ml Erlenmeyer flask (7.2) and add 5 ml of solution 1 (6.2.2). Fit the Erlenmeyer flask with a glass stopper. Stir the solution for 30 min ± 1 min at a temperature of (40 ± 1) °C. Cool the solution in the dark for at least 30 min to a temperature of 18 °C to 26 °C then measure the absorbance spectrophotometrically at 412 nm against a blank solution made from a mixture of 5 ml detergent solution (6.2.1) plus 5 ml solution 1 (6.2.2). Register the absorbance obtained as $E_p$. The maximum time between the end of the derivatization and the measure of the absorbance should be 1 h.

For the purpose of determining the absorbance resulting from the initial colour of the filtrate obtained in 8.2.2, pipette 5 ml of the filtrate (8.2.2) into a 25 ml Erlenmeyer flask (7.2) and add 5 ml of solution 2 (6.2.3). Thereafter, the same method is applied as with the sample. Register the absorbance obtained as $E_e$.

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 0,5 ml of the filtrate (8.2.2) into a 25 ml Erlenmeyer flask (7.2), add 4,5 ml water, then follow the procedure as described in opening paragraph of this subclause.

### 8.2.4 Checking reagents for absence of formaldehyde

Measure 5 ml detergent solution (6.2.1) plus 5 ml solution 1 (6.2.2), using 5 ml of detergent solution (6.2.1) plus 5 ml of water as the reference. The measured absorbance shall not be larger than 0,025 when measured in a 20 mm cell at 412 nm, 0,063 when measured in a 50 mm cell at 412 nm or 0,050 when measured in a 40 mm cell at 412 nm.

### 8.2.5 Testing other compounds which cause a colouring with acetylacetone

Mix 5 ml of the filtrate obtained in 8.2.2 with 1 ml dimedone solution (6.2.4) and warm it up to 40 °C ± 1 °C for (10 ± 1) min. Add 5 ml of solution 1 (6.2.2) and keep the mixture at (40 ± 1) °C for (30 ± 1) min. After cooling down to room temperature, take a spectrophotometer absorbance measurement at 412 nm against a similar solution, which instead of solution 1 (6.2.2) contains 5 ml of solution 2 (6.2.3). This absorbance shall be less than 0,05 (measured in a 20 mm cell) when formaldehyde is found in the leather sample measurement.

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When the absorbance is greater than 0,05, carry out the procedure in accordance with ISO 17226-1. If this is not possible, a note shall be made in the test report to the effect that other compounds were detected within the analysis that could cause a positive response for formaldehyde.

### 8.2.6 Calibration

The following calibration procedure refers to the measurement with a 20 mm cell. Other calibration can be used with a 40 mm cell and 50 mm cell.

Pipette 5 ml of the formaldehyde stock solution obtained in 8.1.1 with an precisely known amount of formaldehyde into a 1 000 ml volumetric flask (7.1), which has been pre-filled with 100 ml water. Mix together and fill the flask up to the mark with water and mix again thoroughly. This solution is the standard solution for calibration purposes, i.e. the standard solution is approximately 10 µg/ml.

From this solution, pipette 1 ml, 5 ml, 10 ml, 15 ml and 20 ml each into separate 50 ml volumetric flasks (7.1) and fill with water. These solutions cover the formaldehyde concentration range of 0,2 µg/ml to 3,0 µg/ml. (This corresponds to a formaldehyde in leather concentration range of 5 mg/kg to 100 mg/kg leather under the given conditions. For higher concentrations, use a smaller aliquot of filtrate.)

From these six solutions, pipette 5 ml of each and mix in a 25 ml Erlenmeyer flask (7.2) with 5 ml solution 1 (6.2.2). Warm this mixture up to (40 ± 1) °C and shake at this temperature for (30 ± 1) min.

After cooling down to room temperature (protect from light), take a spectrophotometer measurement at 412 nm against a blank solution consisting of 5 ml solution 1 (6.2.2) and 5 ml water.

Prior to measuring, set the zero point of the spectrophotometer (7.7) with the blank sample [5 ml solution 1 (6.2.2) and 5 ml water], which was treated under the same conditions as the calibration solutions.

Plot the concentrations in micrograms per millilitre (µg/ml) in a calibration graph against the measured absorbance. *x*-axis: concentration in micrograms per millilitre (µg/ml), *y*-axis: absorbance.

### 8.2.7 Calculation of the content of formaldehyde of the leather sample

$$w_p = \frac{\left(E_p - E_e\right) \times V_o \times V_f}{F \times m \times V_a}$$

where

$w_p$ is the concentration of formaldehyde in the sample in milligrams per kilogram (mg/kg), rounded off to 0,1 mg/kg;

$E_p$ is the absorbance of the filtrate after reaction with acetylacetone;

$E_e$ is the absorbance of the filtrate (initial colour);

$V_o$ is the volume of elution in millilitres (ml) (standard conditions: 50 ml);

$V_a$ is the aliquot taken from the filtrate in millilitres (ml) (standard conditions: 5 ml);

$V_f$ is the volume of solution obtained in 8.2.3 after reaction, in millilitres (ml) (standard conditions: 10 ml);

$F$ is the gradient of calibration curve ($y/x$), in millilitres per microgram (ml/µg);

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

### 8.2.8 Spiking and recovery rate

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

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Pipette 2,5 ml of the filtrate obtained in 8.2.2 into each of two 10 ml volumetric flasks (7.1). Add to one volumetric flask an exactly determined volume of the formaldehyde standard solution for calibration (8.2.6), to give approximately the same concentration as was found in the sample. Fill both volumetric flasks with water to the mark.

If the amount of formaldehyde in the leather is below 20 mg/kg, take a 5 ml aliquot instead of 2,5 ml.

If in the leather sample the amount of formaldehyde is 30 mg/kg, then spike it with 0,5 ml of the formaldehyde standard solution (8.2.6).

Transfer the contents of the volumetric flasks to separate 25 ml Erlenmeyer flasks (7.2). Add 5 ml of solution 1 (6.2.2) and stir for (30 ± 1) min at (40 ± 1) °C.

After cooling down (protect from light), take a measurement of the absorbance at 412 nm against a blank made of 5 ml detergent solution (6.2.1) plus 5 ml solution 2 (6.2.3). Register the absorbance of the spiked sample registered as $E_A$. The absorbance of the unspiked sample is recorded as $E_p$.

$$R_R = \frac{\left(E_A - E_p\right) \times 100}{E_{zu}}$$

where

$E_A$ is the absorbance of the spiked sample;

$E_p$ is the absorbance of the non-spiked sample;

$E_{zu}$ is the expected absorbance for the quantity of formaldehyde that was added (from the calibration graph);

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

If the recovery rate ($R_R$) is not between 80 % and 120 %, the analysis should be repeated.

## 9 Expression of results

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

The measurement precision from an interlaboratory trial with 15 participants is presented in Annex A.

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 17226-2);

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;

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

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