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Leather — Chemical tests — Determination of formaldehyde content

Cuir — Essais chimiques — Détermination de la formaldéhyde

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Foreword

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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/TS 17226 was prepared by the Chemical Test Commission of the International Union of Leather Technologists and Chemists Societies (IUC Commission, IULTCS) in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 289, *Leather*, the secretariat of which is held by UNI, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). This method is technically similar to the method in IUC 19 which was declared an official method at the IULTCS Delegates meeting on 31st May 2003 in Cancun, Mexico. This edition differs slightly in the text compared with IUC 19.

Leather — Chemical tests — Determination of formaldehyde content

1 Scope

This Technical Specification includes two methods for the determination of free and released formaldehyde in leathers. One method (Section 5) is relatively simple with regard to the laboratory equipment (colourimetric method), while the other (Section 4) needs more sophisticated equipment (HPLC method). The second method is selective and not sensitive to coloured extracts.

The formaldehyde content is taken to be the quantity of formaldehyde contained in a water extract from the leather.

The two analytical methods should give similar trends but not necessarily the same absolute result.

2 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 2418:2002, Leather - Chemical, physical and mechanical and fastness tests - Sampling location

ISO 2419:2002, Leather - Physical and mechanical tests - Sample preparation and conditioning

ISO 3696:1987, Water for analytical laboratory use - Specification and test methods

ISO 4044:1977, Leather - Preparation of chemical test samples

3 Preparation and standardisation of formaldehyde stock solution

3.1 Reagents

Unless otherwise stated only analytical grade chemicals are to be used. The water must be demineralised, grade 3 in accordance with ISO 3696. All solutions are aqueous solutions.

3.1.1 Formaldehyde solution, approx. 37 %;

3.1.2 0,05 M iodine solution, (i.e. 12,68 g iodine per l);

3.1.3 2M sodium hydroxide;

3.1.4 1,5 M sulphuric acid;

3.1.5 0,1 M sodium thiosulphate solution;

3.1.6 1 % starch solution, (i.e. 1 g in 100 ml water).

3.2 Procedure for the determination of formaldehyde in the stock solution

5 ml of the formaldehyde solution (3.1.1) is pipetted into a 1 000 ml volumetric flask which contains approximately 100 ml demineralised water and is subsequently filled with demineralised water up to the mark. This solution is the formaldehyde stock solution.

From this solution, 10 ml is pipetted into a 250 ml Erlenmeyer flask and mixed with 50 ml iodine solution (3.1.2) and sodium hydroxide (3.1.3) is added until it turns yellow. It is allowed to settle for 15 min ± 1 min at 18 °C to 26 °C and then 50 ml of sulphuric acid (3.1.4) is added while swirling.

After addition of 2 ml of starch solution (3.1.6), the excess iodine is titrated with sodium thiosulphate (3.1.5) until the colour changes. Three individual determinations shall be made. A blank solution is titrated in the same manner.

$$C_{FA} = \frac{(V_0 - V_1) x\, c_1\, x\, M_{FA}}{2}$$

C_{FA} = concentration of the formaldehyde stock-solution, in mg / 10 ml;

V_0 = titre of the thiosulphate solution for the blank solution, in ml;

V_1 = titre of the thiosulphate solution for the sample solution, in ml;

M_{FA} = molecular weight of formaldehyde, 30,08 g/mol;

c_1 = concentration of the thiosulphate solution, M.

4 Chromatographic HPLC method

4.1 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 water at 40 °C. The eluate is mixed with 2,4 dinitrophenylhydrazine, whereby aldehydes and ketones react to give the respective hydrazines. These are separated by means of a reversed-phase HPLC method, detected at 350 nm and quantified.

4.2 Chemicals

Unless otherwise stated only analytical grade chemicals are to be used. The water must be demineralised, grade 3 in accordance with ISO 3696. All solutions are aqueous solutions.

4.2.1 0,1% sodium dodecylsulphonate (detergent), 1 g in 1 000 ml water;

4.2.2 0,3 % DNPH (2,4 Dinitrophenylhydrazine) in conc. o-Phosphoric acid (85 %). (DNPH recrystallised from 25 % acetonitrile in water);

4.2.3 Acetonitrile.

4.3 Apparatus

4.3.1 Strainer with glass fibre filter, GF8 (or glass filter strainer G 3, diameter 70 - 100 mm);

4.3.2 Water bath, thermostatically controlled to 40 °C ± 0,5 °C, fitted with a flask shaker or stirrer;

4.3.3 Thermometer, with 0,1 °C graduations over the range 20 °C to 50 °C;

4.3.4 HPLC system with UV detection, 350 nm;

4.3.5 Membrane filter, polyamide, 0,45 µm;

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

4.4 Process for the determination of formaldehyde in leather

4.4.1 Sampling and preparation of samples

If possible sample in accordance with ISO 2418:2002 and grind the leather in accordance with ISO 4044:1977. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments) details about sampling have to be given together with the test report.

Condition the ground test sample in accordance with ISO 2419:2002, prior to weighing.

4.4.2 Extraction of formaldehyde

2 g ± 0,1 g leather are weighed into a 100 ml Erlenmeyer flask. 50 ml of the detergent solution (4.2.1) (pre-warmed to 40 °C) is added and the flask is closed with a glass stopper (see Note). The content of the flask is stirred or shaken smoothly at 40 °C ± 0,5 °C in a water bath (4.3.2) for 60 min ± 2 min. The warm extract solution is immediately filtered by vacuum through a glass fibre filter (4.3.1) into a flask. The filtrate, in a closed flask, is cooled down to room temperature (18 °C to 26 °C).

NOTE The leather/solution ratio shall not be modified. Extraction and analysis should be performed within the same working day.

4.4.3 Reaction with DNPH

In a 10 ml volumetric flask pipette 4,0 ml of acetonitrile (4.2.3), a 5,0 ml aliquot of the filtered eluate and 0,5 ml of DNPH solution (4.2.2). The volumetric flask is filled with demineralised water up to the mark and shaken briefly by hand to mix the components. Allow to stand at least 60 min, but not more than a maximum of 180 min. After filtration through a membrane filter (4.3.5) the sample is chromatographed. If the concentration is out of the calibration range smaller aliquots shall be taken. (Standard additions procedure is recommended).

4.4.4 HPLC conditions (recommendation)

Flow rate:	1,0 ml/min
Mobile phase:	acetonitrile / water, 60:40
Separation column:	Merck 100, CH 18,2 (highly coated, 12 % C)+ precolumn (1 cm, RP18)

UV detection wavelength: 350 nm

Injection volume: 20 µl

4.4.5 Calibration of HPLC

0,5 ml of the formaldehyde stock solution (3.2), with an exactly known formaldehyde content, is pipetted into a 500 ml volumetric flask, pre-filled with approximately 100 ml water, mixed, filled with water to the mark, and again mixed. (Content approx. 2 µg formaldehyde /ml).

This solution is the standard solution.

In each of six 10 ml volumetric flasks, add 4 ml acetonitrile (4.2.3) and then add a concentration series of 0,5; 1,0; 2,0; 3,0; 4,0; 5,0 ml respectively of the standard solution. Immediately upon addition of the formaldehyde solution, each flask shall be mixed and 0,5 ml DNPH solution (4.2.2) added. Fill the flasks up to the mark with demineralised water and mix. After at least 60 min and not more than 180 min, the samples shall be chromatographed after filtration through a membrane filter. The calibration is effected through plotting a graph of the formaldehyde derivative peak area versus the concentration in µg/10 ml.

4.4.6 Calculation of the formaldehyde content in leather samples

$$C_F = \frac{C_s x F}{E_W}$$

C_F	concentration of formaldehyde in the sample in mg/kg rounded to 0,01 mg/kg;
C_S	concentration of formaldehyde obtained from the calibration graph in µg/10 ml;
F	dilution factor;
E_W	quantity of leather weighed in g.

4.4.7 Spiking - determination of recovery rate

4 ml acetonitrile are pre-filled into a 10 ml volumetric flask and an aliquot of 2,5 ml of the filtrate, obtained as described in section 4.4.2, added. An accurately determined volume of the formaldehyde standard solution is then added to give an almost equal concentration to that found in the sample.

This solution is further treated analogous to the description under 4.4.5 and C_{S2} determined analogous to section 4.4.3. Carry out the determination in duplicate and report the mean and individual values in the test report.

$$RR = \frac{2x(C_{s2} - C_s)x100}{2xC_{FA1}}$$

C_{S2} = Concentration of formaldehyde obtained from the calibration graph in µg/10 ml;

C_S = Concentration of formaldehyde in the non-spiked sample in µg/10 ml;

C_{FA1} = Spiked quantity of formaldehyde in µg/10 ml;

RR = Recovery rate in percent, rounded to 0,1 %.

5 Colourimetric method

5.1 Scope

The formaldehyde content is taken to be the quantity of formaldehyde contained in the water extract of the leather under the conditions described below referred to the weighed quantity of leather.

This process is not absolutely selective. Detected is not only the free formaldehyde but also the formaldehyde which is hydrolysed during extraction to yield free formaldehyde.

5.2 Principle

The leather sample is eluted with water 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.

5.3 Chemicals

Unless otherwise stated only analytically pure chemicals are to be used. The water must be demineralised, grade 3 in accordance with ISO 3696. All solutions are aqueous solutions.

5.3.1 0,1% sodium dodecylsulphonate (detergent), 1 g in 1 000 ml water;

5.3.2 150 g ammonium acetate + 3 ml glacial acetic acid +2 ml acetylacetone (pentane - 2,4 -dione, CAS 123-54-6) in 1 000 ml water. Prepare fresh daily and keep in a dark place (it is sensitive to light);

5.3.3 150 g ammonium acetate + 3 ml glacial acetic acid in 1 000 ml water;

5.3.4 5 g dimedone in 1 000 ml water (dimedone = Methone = 5,5'-Dimethyl-1,3-cyclohexanedione, CAS 126-81-8).

NOTE It is reported that dimedone can not 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.

5.4 Apparatus

The same apparatus as in 4.3 above.

5.4.1 Spectrophotometer with suitable cells capable of measuring absorbance at 412 nm. The recommended cell path length is 20 mm.

5.5 Preparation and standardisation of formaldehyde stock solution

The same procedure as in Section 3 above.

5.6 Procedure for determination of formaldehyde in leather

5.6.1 Sampling and preparation of samples

If possible sample in accordance with ISO 2418 and grind the leather in accordance with ISO 4044:1977. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments) details about sampling have to be given together with the test report.

Condition the ground test sample in accordance with ISO 2419, prior to weighing.

5.6.2 Extraction of formaldehyde

2 g ± 0,1 g leather are weighed into a 100 ml Erlenmeyer flask. 50 ml of the detergent solution (5.3.1) (pre-warmed to 40 °C) is added and the flask is closed with a glass stopper (see Note). The content of the flask is stirred or shaken smoothly at 40 °C ± 0,5 °C in a water bath (4.3.2) for 60 min ± 2 min. The warm extract solution is immediately filtered by vacuum through a glass fibre filter (4.3.1) into a flask. The filtrate, in a closed flask, is cooled down to room temperature (18 °C to 26 °C).

NOTE The leather/solution ratio shall not be modified. Extraction and analysis should be performed within the same working day.

5.6.3 Reaction with acetylacetone

5 ml of the filtrate obtained in 5.6.2 is pipetted into a 25 ml Erlenmeyer flask and 5 ml of the reagent solution (5.3.2) added. The Erlenmeyer flask is fitted with a glass stopper. The solution is stirred for 30 min ± 1 min at a temperature of 40 °C ± 1 °C. After cooling (in the dark), the absorbance is measured spectrophotometrically at 412 nm against a blank solution made from a mixture of 5 ml detergent solution (5.3.1) + 5 ml reagent solution (5.3.2). The absorbance obtained is recorded as E_p.

For the purpose of determining the absorbance resulting from the initial colour of the filtrate obtained in 5.6.2, 5 ml of the filtrate (5.6.2) is pipetted into a 25 ml Erlenmeyer flask and 5 ml of solution 5.3.3 is added. Thereafter, the same method is applied as with the sample. The absorbance obtained is recorded as E_e.

NOTE Take a smaller aliquot for leathers with a high content of formaldehyde (> 75 mg/kg). Aliquots smaller than 5 ml are made up to 5 ml with water.

Example: formaldehyde content: 500 mg/kg

Procedure: 0,5 ml of the filtrate (5.6.2) is pipetted in a 25 ml Erlenmeyer flask, 4,5 ml water is added. Then follow the procedure as described above.

5.6.4 Checking reagents for absence of formaldehyde

5 ml detergent solution (5.3.1) + 5 ml reagent solution (5.3.2) are measured in relation to 5 ml of detergent solution (5.3.1) + 5 ml of water. The measured absorbance shall not be larger than 0,025 (measured in a 20 mm cell at 412 nm).

5.6.5 Testing other compounds which give a colour with acetylacetone

5 ml of the filtrate obtained in 5.6.2 is mixed with 1 ml dimedone solution (5.3.4) and warmed up to 40 °C ± 1 °C for 10 min ± 1 min. 5 ml of the reagent solution (5.3.2) is added and the mixture is kept at 40 °C ± 1 °C for 30 min ± 1 min. Upon cooling down to room temperature, a spectrophotometer absorbance measurement is taken at 412 nm against a blank solution, which instead of the filtrate (5.6.2) contains 5 ml of the reagent solution (5.3.1). This absorbance must be less than 0,05 (measured in a 20 mm cell) when formaldehyde is found in the leather sample.

5.6.6 Calibration

3 ml of the formaldehyde stock solution obtained in 3.2 with an exactly known amount of formaldehyde is pipetted into a 1 000 ml volumetric flask, which has been pre-filled with 100 ml water. They are mixed together and the flask filled up to the mark with water and again thoroughly mixed. This solution is the standard solution for calibration purposes (i.e. the standard solution is approx. 6 µg / ml).

From this solution 3, 5, 10, 15, 25 ml aliquots are pipetted into separate 50 ml volumetric flasks and filled with water. These solutions then cover the formaldehyde concentration range of 0,4 µg/ml to 3,0 µg/ml. (This corresponds to a formaldehyde in leather concentration range of 9 mg / kg to 75 mg / kg leather under the given conditions. For higher concentrations a smaller aliquot of filtrate is used).

From each of these 5 solutions, 5 ml is pipetted and mixed in a 25 ml Erlenmeyer flask with 5 ml reagent solution (5.3.2). This mixture is intensively shaken and warmed up to 40 °C ± 1 °C for 30 min ± 1 min.

Upon cooling down to room temperature (protect against light) a spectrophotometer measurement is taken at 412 nm against a blank solution consisting of 5 ml reagent solution (5.3.2) and 5 ml water.

Prior to measuring, the zero point of the spectrophotometer is set with the blank sample (5 ml reagent solution (5.3.2) and 5 ml water), which was treated under the same conditions as the calibration solutions.

The concentrations in µg/ml are plotted in a calibration graph against the measured absorbance, x-axis: concentration in µg/ml, y-axis: absorbance.

5.6.7 Calculation of the content of formaldehyde of the leather sample

$$C_p = \frac{(E_p - E_e) x V_0 x V_f}{F x W x V_a}$$

C_p = Concentration of formaldehyde in the sample in mg/kg, rounded off to 0,1 mg / kg

E_p = Absorbance of filtrate after reaction with acetylacetone

E_e = Absorbance of filtrate (initial colour)

V_0 = Volume of elution in ml *(standard conditions: 50 ml)*

V_a = Aliquot taken from filtrate in ml *(standard conditions: 5 ml)*

V_f = Volume of solution obtained in 5.6.3 after reaction, in ml *(standard conditions: 10 ml)*

F = gradient of calibration curve (y/x) in ml/µg

W = weight of leather in g

5.6.8 Spiking and Recovery Rate

2,5 ml (see Note 1) of the filtrate obtained in 5.6.2 is pipetted into two 10 ml volumetric flasks. Add to one volumetric flask an exactly determined volume of the formaldehyde standard solution for calibration (5.6.6) to give approximately the same concentration as was found in the sample (see Note 2). Both volumetric flasks are filled with water to the mark.

The contents of the volumetric flasks are then transferred to separate 25 ml Erlenmeyer flasks, 5 ml of reagent solution (5.3.2) added to each and stirred for 30 min ± 1 min at 40 °C ± 1 °C.

After having cooled down (protect from light), a measurement of the absorbance is taken at 412 nm against a blank made of 5 ml detergent solution (5.3.1) + 5 ml reagent solution (5.3.2). The absorbance of the spiked sample is recorded as E_A. The absorbance of the unspiked sample is recorded as E_P.

NOTE 1 If the amount of formaldehyde in the leather is below 20 mg/kg, take a 5 ml aliquot instead of 2,5 ml.

NOTE 2 Example: If in the leather sample there is 30 mg / kg then spike with 0,5 ml of the formaldehyde standard solution (5.6.6).

$$RR = \frac{2x(E_A - E_P)x100}{2x E_{zu}}$$

E_A = absorbance of the spiked sample;

E_P = absorbance of the non-spiked sample;

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

RR = recovery rate in per cent , rounded off to 0,1 %.