INTERNATIONAL STANDARD

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IULTCS/IUC 29

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Leather — Chemical tests — Determination of the preservative (TCMTB, PCMC, OPP, OIT) content in leather by liquid chromatography

Cuir — Essais chimiques — Dosage des agents de conservation (TCMTB, PCMC, OPP, OIT) dans le cuir par chromatographie en phase liquide

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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 13365 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 method in IUC 29.

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.

Leather — Chemical tests — Determination of the preservative (TCMTB, PCMC, OPP, OIT) content in leather by liquid chromatography

1 Scope

This International Standard specifies a test method for the determination of the content of the following preservative agents:

— 2-(thiocyanomethylthio)-benzothiazole (TCMTB);

— 4-chloro-3-methylphenol (PCMC);

— 2-phenylphenol (OPP);

— 2-octylisothiazol-3(2H)-one (OIT);

in leather by liquid chromatography.

Preservative agents are necessary to protect leather from microbiological attack.

NOTE The preservative agents 4-chloro-3-methylphenol (PCMC) and 2-phenylphenol (OPP) can also be determined according to ISO 17070 and quantified by means of gas chromatography/mass spectroscopy (GC/MS).

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

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

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

3 Principle

The leather sample is extracted with a suitable solvent using ultrasonic waves. The filtered extract is analysed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

4 Reagents

4.1 TCMTB, minimum 99,7 %.

4.2 TCMTB stock solution, 500 mg/l in acetonitrile.

4.3 **PCMC**, minimum 99,5 %.

4.4 **PCMC stock solution**, 500 mg/l in acetonitrile.

4.5 **OPP**, minimum 99,5 %.

4.6 **OPP stock solution**, 500 mg/l in acetonitrile.

4.7 **OIT**, minimum 97,0 %.

4.8 **OIT stock solution**, 500 mg/l in acetonitrile.

4.9 **Acetonitrile**, HPLC grade.

4.10 **Water**, HPLC grade.

5 Apparatus and materials

Usual laboratory apparatus is required and, in particular, the following.

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

5.2 **HPLC system**, with UV detection or other suitable detectors.

5.3 **Separation column**, reversed phase C8 or C18 with corresponding pre-column.

5.4 **Ultrasonic bath**, e.g. 40 kHz.

5.5 **Membrane filter**, polyamide, 0,45 µm.

6 Procedure

6.1 Sampling and sample preparation

If possible, sample in accordance with ISO 2418 and grind in accordance with ISO 4044. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products, such as shoes or garments), details of the sampling shall be given, together with the test report.

Wet intermediates like wet blue and wet white shall be dried as described in ISO 4044 prior to grinding.

6.2 Preparation of analytical solution

Weigh (1 ± 0,01) g of ground leather to the nearest 0,001 g in a 100 ml conical flask. Pipette 20 ml of acetonitrile (4.9) and add it to the leather. The leather sample is extracted in an ultrasonic bath (5.4) for 1 h ± 5 min (80 % ultrasound power) at room temperature. During extraction, the temperature in the mixture increases to about 35 °C.

Subsequently, a part of the extract is filtered through a membrane filter (5.5) into a suitable vial.

The filtrate is analysed by HPLC (5.2) and detected preservatives are quantified.

6.3 Chromatographic conditions

Proposal:

Separation column:	reversed-phase (RP) column (C18 HD, 250 mm/4 mm, 100 Å, 5 µm) with pre-column
Flow rate:	0,8 ml/min
Mobile phase:	A: water, B: acetonitrile
Gradient:	60 % B for 6 min isocratic, then linear to 95 % B in 9 min
Column oven:	30 °C
UV detection:	275 nm
Injection volume:	20 µl

6.4 Calibration

Calibration is carried out by means of an external standard. Prepare adequate dilutions (in acetonitrile) of preservative stock solutions (4.2, 4.4, 4.6 and 4.8). Calibration shall be done using at least six concentration levels. The calibration is effected by plotting a graph of the preservative peak area versus its concentration, in micrograms per millilitre (µg/ml).

As very different preservative concentrations can be expected, it is not possible to cover the whole range with a single calibration curve.

7 Calculation

Calculate the mass fraction, w_i, of every preservative detected, in milligrams per kilogram (mg/kg) of leather, using the following equation:

$$w_i = \frac{\rho \cdot V \cdot F \times 1000}{m \times 1000}$$

where

w_i is the mass fraction, expressed in milligrams per kilogram (mg/kg), of a certain preservative in leather;

ρ is the mass concentration of preservative obtained from the calibration, in micrograms per millilitre (µg/ml);

V is the extract volume, in millilitres (ml);

F is the dilution factor;

m is the quantity of sample weighed, in grams (g).

The mass fraction of each preservative is given in milligrams per kilogram (mg/kg), rounded to the nearest 0,1 mg.

The mass fraction of preservative referred to dry matter, according to ISO 4684, can be calculated using the following equation:

$$w_{dm} = w \cdot D$$

where

w_{dm} is the mass fraction of preservative, expressed in milligrams per kilogram (mg/kg), of sample referred to dry matter;

w is the mass fraction of preservative, expressed in milligrams per kilogram (mg/kg), of the sample being tested;

D is the recalculation factor for dry matter

$$D = \frac{100}{100 - w_v}$$

w_v is the mass fraction of volatile matter, based on ISO 4684, expressed as a percentage.

8 Test report

The test report shall include the following information:

a) a reference to this International Standard, i.e. ISO 13365:2011;

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

c) the type of liquid chromatography detection;

d) the analytical result for each mass fraction of preservative, in milligrams per kilogram (mg/kg) rounded to one decimal place;

e) any deviation from the analytical procedure.