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

Part 1: Acetonitrile extraction method

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

Partie 1: Extraction à l'acétonitrile

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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 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).

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 first edition of ISO 13365-1, together with ISO 13365-2, cancels and replaces ISO 13365:2011, which has been technically revised and split into two parts. The main changes in ISO 13365-1 from ISO 13365:2011 are as follows:

— the title has been changed to indicate the method of extraction;

— the use of HPLC with mass spectrometric (MS) detection has been included;

— Clause 5 (former Clause 4) has been technically modified. In addition, the calibration information (previously in 6.4) is now included in Clause 5;

— the chromatographic conditions (previously in 6.3) are now included in a new Annex A. Annex A also includes additional conditions for use with MS detection.

A list of all parts in the ISO 13365 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 the preservative (TCMTB, PCMC, OPP, OIT) content in leather by liquid chromatography —

Part 1:
Acetonitrile extraction method

1 Scope

This document specifies a test method by acetonitrile solvent extraction for the determination of the total content (solvent extractible) of the following preservative agents in leather by liquid chromatography:

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

— 4-chloro-3-methylphenol (PCMC);

— 2-phenylphenol (OPP);

— 2-octylisothiazol-3(2H)-one (OIT);

This method can also be used to determine breakdown products of these preservative agents, which protect leather from microbiological attack.

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 Principle

Extraction of the leather sample is performed with a suitable solvent using ultrasonic waves. The filtered extract is analysed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) or mass spectrometry (MS) detection.

5 Reagents

5.1 General

The chemicals in Table 1 and Table 2 shall be used in the defined purity, as follows:

— above 95 % for the pure product used for the reference target compounds;

— HPLC grade for the solvent;

— deionized water (at least grade 3).

Table 1 — Reagents for extraction

Chemical	Purity
Acetonitrile, CAS 75-05-8	HPLC Grade
Deionized water	≥ grade 3, ISO 3696

Table 2 — Reagents for LC-MS or LC-UV analysis

Chemical	Purity
Deionized water	≥ grade 3, ISO 3696
Acetonitrile, CAS 75-05-8	HPLC grade
Formic acid, CAS 64-18-6	Analytical grade
Methanol, CAS 67-56-1	LC-MS grade
Ammonium bicarbonate, CAS 1066-33-7	Analytical grade
TCMTB, CAS 21564-17-0	Minimum 99,7 %.
PCMC, CAS 59-50-7	Minimum 99,5 %.
OPP, CAS 90-43-7	Minimum 99,5 %.
OIT, CAS 26530-20-1	Minimum 97,0 %.

5.2 Target compounds

Prepare a solution to 100 mg/l of each target compound (TCMTB, PCMC, OPP, OIT) or use a commercial solution.

5.3 Preparation of calibration solutions

At least four calibration solutions shall be prepared in a range of concentrations (C), see Table 3, to match the limits given. As very different preservative concentrations can be expected, it is not possible to cover the whole range with a single calibration curve.

Prepare suitable calibration solutions using acetonitrile (Table 2) and target compound solutions (5.2) according to the volumes (V) given in Table 3.

Table 3 — Calibration solutions

Values in µl

Calibration solution	C				
	2 mg/l	10 mg/l	25 mg/l	50 mg/l	100 mg/l
V acetonitrile	980	900	750	500	0
V target compound	20	100	250	500	1 000

Calibration solutions shall be stored at (4 ± 3) °C.

6 Apparatus and materials

The usual laboratory apparatus is required and, in particular, the following.

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

6.2 HPLC system, with UV or MS detection, or other suitable detectors.

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

6.4 Ultrasonic bath, for example 40 kHz.

6.5 Membrane filter, coupled to a 10 ml syringe, polyamide, 0,45 µm.

7 Testing procedure

7.1 Sampling and sample preparation

Sample in accordance with ISO 2418 and prepare in accordance with ISO 4044. If sampling in accordance with ISO 2418 is not possible (for example, 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 preparing the samples.

7.2 Extraction procedure

Weigh approximately 1 g of prepared leather to the nearest 0,001 g in a 100 ml conical flask. Pipette 20 ml of acetonitrile (Table 1) and add it to the leather. Perform the extraction of the leather sample in an ultrasonic bath (6.4) for 1 h ± 5 min at room temperature. During extraction, the temperature in the mixture increases to about 35 °C.

If necessary, an aliquot of the extract can be filtered through a membrane filter (6.5) into a suitable vial or centrifugated.

7.3 Analytical procedure

Analyse the extract using HPLC (6.2).

Various types of HPLC equipment with UV or MS detector can be used. Guidelines for suitable chromatographic conditions are given in Annex A.

Use the calibration solutions (5.3) to obtain a linear calibration curve of peak area vs concentration in µg/l.

8 Calculation

Calculate the mass fraction, w_i, of every preservative detected, in milligrams per kilogram (mg/kg) of leather, rounded to the nearest 0,1 mg, using Formula (1):

$$w_i = (\rho \cdot V \cdot F)/m \quad (1)$$

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 mass of sample weighed, in grams (g).

If required, calculate the mass fraction of preservative referred to dry matter, according to ISO 4684, using Formula (2):

$$w_{dm} = w \cdot D \quad (2)$$

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, which is calculated according to the following formula:

$$D = 100/(100 - w_v)$$

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

This method allows the quantification of a minimum concentration of 10 mg/kg.

9 Test report

The test report shall include the following information:

a) a reference to this document, i.e. ISO 13365-1:2020;

b) identification of the sample and, if necessary, details of sampling;

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;

f) the date of the test.

Annex A
(informative)

Chromatographic conditions

A.1 Overview

Various types of high-performance liquid chromatographic equipment with UV or mass spectrometric detector (LC-MS) can be used. The chromatographic equipment, column and operating conditions in A.2 and A.3 are examples of equipment and parameters that have been found suitable for this analysis.

A.2 HPLC with UV detector

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

A.3 HPLC with MS detector

A.3.1 LC-MS for PCMC and OPP

Separation column:	reversed-phase (RP) column (C18 HD, 250 mm/4 mm, 100 Å, 5 µm) with pre-column
Flow rate:	0,7 ml/min
Mobile phase:	A: water and ammonium bicarbonate (10 mM), B: methanol
Gradient:	50 % B to 80 % B in 15 min in gradient mode
Column oven:	40 °C
Injection volume:	10 µl
SIM negative:	m/z 169 OPP m/z 141 PCMC
Nebulizing gas flow:	1,5 l/min
Drying gas flow:	10 l/min