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Leather — Determination of total content of certain bisphenols

Cuir — Détermination de la teneur totale en certains bisphénols

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Contents

Page

Foreword

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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 document 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).

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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 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*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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.

Introduction

This document includes a procedure for analysing certain bisphenols using liquid chromatography (LC) equipment. With this analytical method, bisphenol A, bisphenol B, bisphenol F and bisphenol S can be determined.

In the leather industry, bisphenol F can be an impurity in synthetic tanning agents. Bisphenol S is a monomer that is used to manufacture synthetic tanning agents, which can lead to residues in the final product.

Bisphenol A is an endocrine disruptor for environmental organisms. Bisphenol A is a synthetic organic chemical primarily used as a monomer in the manufacture of high-performance plastics, other polymers, such as resins, and in the colour developer for thermoprint paper. Bisphenol B is similar to bisphenol A and is used in the manufacture of plastics and resins.

Leather — Determination of total content of certain bisphenols

1 Scope

This document specifies a method for determining the total content (solvent extractible) of the following bisphenols in leather:

— bisphenol A;

— bisphenol B;

— bisphenol F;

— bisphenol S.

This method requires the use of liquid chromatography (LC) with either a single quadrupole mass spectrometer (MS), a triple quadrupole mass spectrometer (MS/MS), an ultraviolet (UV) detector, a diode array detector (DAD) or a fluorescence detector (FLD) to identify and quantify the bisphenols.

NOTE This method can also be used for other bisphenols if they are validated by the laboratory.

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, mechanical and fastness tests — Position and preparation of specimens for testing*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

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

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

The leather sample is extracted in methanol using an ultrasonic bath. Subsequently, an aliquot of the solution can be directly analysed, without further cleaning of the sample, using LC-MS, LC-MS/MS or LC with a UV detector (LC-UV), DAD (LC-DAD) or FLD (LC-FLD).

5 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

5.1 Ultrasonic bath, with controllable heating capable of maintaining a temperature of (60 ± 5) °C.

5.2 Glass container with a screw cap, e.g. volume of 20 ml.

5.3 Suitable syringe membrane filters, e.g. polyamide with pore size 0,2 µm.

5.4 Volumetric flasks, e.g. volume of 10 ml and 100 ml.

5.5 LC vials, with cap, e.g. volume of 2 ml.

5.6 Analytical balance, with a resolution of 1 mg or better.

5.7 Pipettes, various sizes, e.g. volume of 1 ml to 5 ml.

5.8 Instrumental equipment, LC-MS/MS.

5.9 Alternative instrumental equipment, LC-MS, LC-UV, LC-DAD or LC-FLD.

NOTE If two detectors are used, they can be arranged in series on the same LC system.

6 Reagents

If not otherwise specified, analytical reagent grade chemicals shall be used.

6.1 Methanol, CAS Registry Number® (CAS RN®)[1)] 67-56-1, for LC-MS/MS it is necessary to have LC-MS quality.

6.2 Water, deionised or distilled water, grade 3 according to ISO 3696.

6.3 Bisphenol A, CAS RN® 80-05-7.

6.4 Bisphenol B, CAS RN® 77-40-7.

6.5 Bisphenol F, CAS RN® 620-92-8.

6.6 Bisphenol S, CAS RN® 80-09-1.

6.7 Stock solutions of a mix of bisphenol A, B, F and S, ρ = 1 mg/l and 10 mg/l.

EXAMPLE 10 mg of each of the respective bisphenols, A (6.3), B (6.4), F (6.5) and S (6.6), is dissolved in separate 100 ml volumetric flasks (5.5) with methanol (6.1). Mixed stock solutions are prepared to obtain, respectively, concentrations of 1 mg/l and 10 mg/l in methanol.

6.8 Internal standard, ρ = 50 mg/l.

When using LC-MS or LC-MS/MS, the use of internal standards for each type of bisphenol is highly recommended to avoid matrix effects.

Examples of suitable mass-labelled internal standards:

1) CAS Registry Number® (CAS RN®) is a trademark of CAS Corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

for bisphenol A: bisphenol A-D8, CAS RN® 92739-58-7;

bisphenol A-D16, CAS RN® 96210-87-6;

for bisphenol B: bisphenol B-D8, CAS RN® to be assigned;

for bisphenol F: bisphenol F-D10, CAS RN® 1794786-93-8;

for bisphenol S: bisphenol S-D8, CAS RN® 2483831-28-1.

Prepare a 50 mg/l solution of the internal standard by diluting the commercial solution with methanol.

6.9 Calibration solutions of bisphenols.

Prepare at least four calibration solutions of ρ = 20 µg/l to ρ = 1 000 µg/l of bisphenols using the stock solutions (6.7), see Table 1. Internal standard is only added if the MS technique is used for detection. In other cases, the internal standard volume is replaced by methanol.

Table 1 — Example of calibration solutions for LC-MS/MS

Concentration µg/l	Volume methanol µl (6.1)	Volume of mix of bisphenols 1 mg/l µl (6.7)	Volume of mix of bisphenols 10 mg/l µl (6.7)	Volume of internal standard at 50 mg/l µl (6.8) (only for MS detection)
20	960	20		20
50	930	50		20
100	880	100		20
200	960		20	20
500	930		50	20
1 000	880		100	20

7 Preparation of test sample and test pieces

The leather test piece shall be taken according to ISO 2418. If a test piece according to ISO 2418 is not possible (e.g. in the case of leather from finished products such as shoes and clothing), the details of how the test piece is taken shall be given in the test report.

The leather test piece shall be cut into small pieces according to ISO 4044.

8 Procedure

8.1 Extraction

Weigh (0,5 ± 0,05) g of the test specimen measured with an accuracy of 10 mg with an analytical balance (5.6) in a screw-top glass container (5.2) and add 10 ml methanol (6.1). Close the container and place it for (60 ± 5) min in an ultrasonic bath (5.1) at (60 ± 5) °C.

After cooling down to room temperature, an aliquot of the extraction solution is filtered (5.3) into a LC sample vial (5.5). The aliquot is now ready for the LC-UV, LC-DAD or LC-FLD analysis.

For LC-MS or LC-MS/MS, after cooling down to room temperature, 200 µl of the extraction solution is filtered (5.3) into a LC sample vial (5.5); 780 µl of methanol (6.1) and 20 µl of internal standard (6.8) are added. The aliquot is now ready for the LC-MS or LC-MS/MS analysis.

8.2 Instrumental analysis

The detection of the bisphenols is made using LC-MS/MS (5.8) or, alternatively, LC-MS, LC-UV, LC-DAD or LC-FLD (5.9). Examples of suitable chromatographic conditions are given in Annex A (for LC-MS/MS), Annex B (for LC-UV, LC-DAD and LC-FLD) and Annex C (for LC-MS).

If the concentration of bisphenols is out of the range of the calibration, make a suitable dilution and inject the new aliquot.

9 Expression of results

9.1 Calculation without internal standard

The content of each bisphenol is calculated as the mass fraction, *w*, in milligrams per kilogram (mg/kg) of the leather sample according to Formula (1):

$$w = \frac{(A_s - b) \cdot V}{a \cdot m} \tag{1}$$

where

A_s is the peak area of each bisphenol in the extraction solution;

b is the intercept of the calibration graph;

a is the slope of the calibration graph;

V is the final volume used (0,01 l);

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

If required, the results can be given based on the dry mass of the leather sample. Details shall be noted in the test report.

9.2 Calculation with internal standard

The content of each bisphenol is calculated as the mass fraction, *w*, in milligrams per kilogram (mg/kg) of the leather sample according to Formula (2):

$$w = \left(\frac{V \cdot d}{m}\right) \cdot \frac{\left(\frac{A_s}{A_{isample}} - b\right) \cdot C_{isample}}{a} \tag{2}$$

where

A_s is the peak area of each bisphenol in the extraction solution;

$A_{isample}$ is the peak area of the corresponding internal standard in the extraction solution;

$C_{isample}$ is the concentration of the corresponding internal standard in the extraction solution in micrograms per litre (µg/l);

b is the intercept of the calibration graph;

d is the dilution factor (here d = 5 for dilution 200 µl to 1 000 µl);

a is the slope of the calibration graph;

V is the final volume used (0,01 l);

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

9.3 Calculation of the results of a sum

The result may be expressed as a sum of different bisphenols.

All the bisphenols included in the sum shall be clearly identified.

The results of the relevant identified bisphenols (9.1 or 9.2) are added to give the result of the sum.

For LC-MS/MS or LC-MS, if the result for a single bisphenol is lower than 5 mg/kg, this result is considered as zero and is not included in the sum.

For LC-UV, LC-DAD or LC-FLD, if the result for a single bisphenol is lower than the quantification limit, this result is considered as zero and is not included in the sum.

10 Precision

With this method it is feasible to reach limits of quantification (LoQ) below 10 mg/kg for each bisphenol with LC-MS/MS and 100 mg/kg with LC-UV, LC-DAD or LC-FLD.

The results of interlaboratory trials to determine the bisphenol S and bisphenol F content in leather are presented in Annex D.

11 Test report

The test report shall include at least the following information:

a) a reference to this document, i.e. ISO 11936:2023;

b) type, origin and description of the leather sample, if possible;

c) date of the test;

d) the type of LC equipment used;

e) mass fraction of each quantified bisphenol (9.1 or 9.2) and the sum (9.3), if requested, expressed in milligrams per kilogram (mg/kg);

f) any deviations from the procedure;

g) any unusual features observed.

Annex A
(informative)

Chromatographic analysis operating parameters for LC-MS/MS

A.1 Preliminary comment

As the LC equipment (5.8) of the laboratories can vary, no general valid instructions can be provided for the chromatographic analysis. The following parameters have been successfully tested and used.

A.2 LC-MS/MS operating parameters

A.2.1 LC-MS/MS chromatographic conditions

Eluent 1: ultrapure water

Eluent 2: methanol

Stationary phase: reverse-phase C18 column, 150 mm × 3,0 mm, 3 µm, 22 % carbon load, with C18 guard column

Column temperature: 35 °C

Injection volume: 2 µl

Volume flow: 400 µl/min

Gradient: see Table A.1

Detection parameters: see Table A.2

Table A.1 — Gradient programme

Time min	Eluent 1 %	Eluent 2 %
0	55	45
12	10	90
12,5	55	45
19	55	45

Detection mode: multiple reaction monitoring (MRM)

Collision gas: nitrogen

Spray gas: nitrogen

Ionization: electrospray ion source (ESI) in negative mode

Drying gas temperature: 300 C

Drying gas flow: 6 l/min

Sheath gas temperature: 320 °C

Sheath gas flow: 11 l/min

Nebulizer pressure: 35 psi

Capillary voltage: 2 000 V

A.2.2 Typical ions for LC-MS/MS

Table A.2 — Typical ions for LC-MS/MS

Bisphenol compounds	**MRM** m/z		**Declustering potential** V	**Collision energy** eV
	Precursor ion	**Product ion**		
bisphenol A	227	212[a]	100	20
		133[b]	100	20
bisphenol B	241	212[a]	140	10
		211[b]	140	20
bisphenol F	199	93[a]	100	20
		105[b]	100	20
bisphenol S	249	108[a]	160	25
		92[b]	160	35

[a] Quantification ion.

[b] Qualification ion

Annex B
(informative)

Chromatographic analysis operating parameters for LC-UV, LC-DAD or LC-FLD

Eluent 1: ultrapure water

Eluent 2: acetonitrile

Stationary phase: reverse-phase C18 column, 100 mm × 2,1 mm, 2,7 µm

Column temperature: 40 °C

Injection volume: 2 µl

Volume flow: 400 µl/min

Gradient: see Table B.1

Detection parameters: see Table B.2

Table B.1 — Gradient programme

Time min	Eluent 1 %	Eluent 2 %
0	95	5
4	80	20
6	80	20
8	50	50
9	5	95
11	5	95
11,5	95	5
13,5	95	5

Table B.2 — Detection wavelengths for UV, DAD and FLD

Bisphenol compounds	Detectors	Wavelength λ
bisphenol S/bisphenol B	UV, DAD	259 nm
bisphenol F	UV, DAD	230 nm
bisphenol F	FLD	excitation: 228 nm emission: 301 nm
bisphenol A	FLD	excitation: 275 nm emission: 313 nm