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Chemicals for the leather tanning industry — Determination of the total content of certain bisphenols

Produits chimiques pour l'industrie du tannage du cuir — Détermination de la teneur totale en certains bisphénols

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Foreword

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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).

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Introduction

This document includes a procedure for analysing certain bisphenols in leather tanning chemicals using liquid chromatography (LC) equipment. With this analytical method, bisphenol A, bisphenol AF, 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 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 AF is a fluorinated organic compound that is an analogue of bisphenol A in which the two methyl groups are replaced with trifluoromethyl groups. Bisphenol B is similar to bisphenol A and is used in the manufacture of plastics and resins.

At present, the official European Chemicals Agency (ECHA) classification recognized in the European Union (EU) is the following:

- bisphenol A as toxic to reproduction, skin sensitizer and endocrine disruptor;[1]
- bisphenol B as endocrine disruptor;[2]
- bisphenol S as toxic to reproduction and endocrine disruptor.[3]

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Chemicals for the leather tanning industry — Determination of the total content of certain bisphenols

1 Scope

This document specifies a method for determining the total content (solvent extractable) of the following bisphenols in chemicals for the leather tanning industry:

- bisphenol A;
- bisphenol AF;
- 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 1 This method can also be used for other bisphenols if they are validated by the laboratory.

NOTE 2 Bisphenol S cannot be detected with FLD.

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2 Normative references

There are no normative references in this document.

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 sample of the chemical is extracted in methanol using an ultrasonic bath. Subsequently, an aliquot of the solution can be directly analysed, 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, for example volume of 20 ml.

5.3 Suitable syringe membrane filters, for example PTFE with pore size 0,2 µm or PTFE-with pore size 0,45 µm.

5.4 Volumetric flasks, for example volume of 10 ml and 100 ml.

5.5 LC vials, with cap, for example volume of 2 ml.

5.6 Analytical balance, with a resolution of 0,1 mg.

5.7 Pipettes, various sizes, for example volume of 1 ml to 20 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.

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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. HPLC quality methanol is suitable for LC-UV, LC-DAD or LC-FLD.

6.2 Water, deionised or distilled, ultra-pure quality for LC-MS and LC-MS/MS, HPLC grade for LC-DAD, UV and FLD.

6.3 Bisphenol A, CAS RN 80-05-7, minimum 98,0 %.

6.4 Bisphenol AF, CAS RN 1478-61-1, minimum 98,0 %.

6.5 Bisphenol B, CAS RN 77-40-7, minimum 98,0 %.

6.6 Bisphenol F, CAS RN 620-92-8, minimum 98,0 %.

6.7 Bisphenol S, CAS RN 80-09-1, minimum 98,0 %.

6.8 Stock solutions of a mix of bisphenol A, AF, B, F and S, ρ = 1 mg/l, 10 mg/l and 50 mg/l.

EXAMPLE 100 mg of each of the respective bisphenols, A (6.3), AF (6.4) B (6.5), F (6.6) and S (6.7), is dissolved in separate 100 ml volumetric flasks (5.4) with methanol (6.1). Mixed stock solutions are prepared to obtain, respectively, concentrations of 1 mg/l, 10 mg/l and 50 mg/l in methanol.

6.9 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:

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[1] Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). 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.

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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 AF:	bisphenol AF-13C12, 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.

6.10 Calibration solutions of bisphenols.

For LC-MS/MS or LC/MS techniques, prepare at least four calibration solutions of ρ = 0,05 µg/ml to ρ = 1 µg/ml of bisphenols using the stock solutions (6.8), see Table 1. For LC-DAD or UV or FLD techniques, prepare at least four calibration solutions of ρ = 0,5 µg/ml to ρ = 20 µg/ml using the stock solutions (6.8), see Table 2.

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

Concentration µg/ml	Volume methanol (6.1) µl	Volume of mix of bisphenols 1 mg/l (6.8) µl	Volume of mix of bisphenols 10 mg/l (6.8) µl	Volume of internal standard at 50 mg/l (6.9) (only for MS detection) µl
0,05	930	50	0	20
0,1	880	100	0	20
0,2	960	0	20	20
0,5	930	0	50	20
1	880	0	100	20

Table 2 — Example of calibration solutions for LC-DAD or UV or FLD

Concentration µg/ml	Volume methanol (6.1) µl	Volume of mix of bisphenols 10 mg/l (6.8) µl	Volume of mix of bisphenols 50 mg/l (6.8) µl
0,5	950	50	0
2	800	200	0
10	0	1 000	0
20	600	0	400

7 Sampling and sample preparation

The sample should be thoroughly mixed to get a representative test portion for analysis.

In the case of a powdered sample, if the particles are heterogeneous, manually or mechanically mill to homogenize the size of the particles.

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8 Procedure

8.1 Extraction

Accurately weigh (0,2 ± 0,050) g of the sample measured to the nearest 0,001 g with an analytical balance (5.6) in a screw-top glass container (5.2) and add 20 ml methanol (6.1). Close the container and place it for (60 ± 5) min in a pre-heated ultrasonic bath (5.1) at (60 ± 5) °C.

After cooling down to room temperature, if the extraction solution appears opalescent or contains particles, centrifugate it for (5 ± 1) min at (4 000 ± 400) rpm, then filter an aliquot of extraction solution (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, take an aliquot of 200 µl of filtered extraction solution (5.3) into a LC sample vial (5.5). Add 780 µl of methanol (6.1) and 20 µl of internal standard (6.9). The aliquot is now ready for the LC-MS or LC-MS/MS analysis.

NOTE Due to the composition of the matrix, it is possible that at the end of the extraction procedure the solution will be opalescent, due to the low solubility in methanol of the other components of the chemical product analysed. This does not affect the extraction efficiency of bisphenols as they are very soluble in the extraction solution.

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-MS) and Annex C (for LC-UV, LC-DAD and LC-FLD).

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 chemical sample according to Formula (1):

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

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 (20 ml);

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

Any detail shall be noted in the test report.

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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 chemical sample according to Formula (2):

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

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 millilitre (µg/ml);
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 (20 ml);
m	is the mass of the chemical sample in grams (g).

9.3 Calculation of the results as 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 the limit of quantification (see Clause 10), 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 limit of quantification (see Clause 10), 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 LC-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 chemicals 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 21135:2024;

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

c) date of the test;

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d) the type of detection technique 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.

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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.

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A.2 LC-MS/MS operating parameters

A.2.1 LC-MS/MS chromatographic conditions

Eluent 1:	ultrapure water for LC-MS/MS
Eluent 2:	methanol for LC-MS/MS
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 AF	335	265[a]	140	20
		69[b]	140	25
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.

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Annex B
(informative)

Chromatographic analysis operating parameters for LC-MS

Eluent 1:	ultrapure water for LC-MS
Eluent 2:	methanol for LC-MS
Stationary phase:	reverse-phase C18 column, 100 mm × 2,1 mm, 2,7 µm
Column temperature:	40 °C
Injection volume:	2 µl
Volume flow:	0,8 ml/min
Gradient:	see Table B.1
Detection parameters:	see Table B.2

Table B.1 — Gradient programme

Time min	Eluent 1 %	Eluent 2 %
0,01	70	30
20,00	20	80
21,00	20	80
25,00	70	30
28,00	stop	stop

Table B.2 — Typical ions for LC-MS

Bisphenol compounds	m/z target ion	m/z reference ion
bisphenol S 4,4’	249 (-)	499 (-)
bisphenol S 2,4’	249 (-)	499 (-)
bisphenol F	199 (-)	231 (-)
bisphenol A	227 (-)	259 (-)
bisphenol B	241 (-)	273 (-)
bisphenol AF	335 (-)	367 (-)

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Annex C
(informative)

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

Eluent 1:	ultrapure water for HPLC
Eluent 2:	acetonitrile for HPLC
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 C.1
Detection parameters:	see Table C.2

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

Bisphenol compounds	Detectors	Wavelength λ
bisphenol A	UV, DAD	225 nm, 278 nm
bisphenol S/bisphenol B	UV, DAD	259 nm
bisphenol F	UV, DAD	228 nm, 278 nm
bisphenol AF	UV, DAD	228 nm, 273 nm
bisphenol F/bisphenol B	FLD	excitation: 265 nm emission: 305 nm
bisphenol A	FLD	excitation: 275 nm emission: 313 nm

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