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Leather — Determination of chlorinated hydrocarbons in leather —

Part 2:
Chromatographic method for middle-chain chlorinated paraffins (MCCPs)

Cuir — Dosage des hydrocarbures chlorés dans le cuir —

Partie 2: Méthode chromatographique pour les paraffines chlorées à chaîne moyenne (PCCM)

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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).

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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.

A list of all parts in the ISO 18219 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.

Introduction

Middle-chain chlorinated paraffins (MCCPs) are a mixture of chlorinated hydrocarbons with a carbon chain length of 14 to 17, and a chlorine content range of 40 % to 70 %. MCCPs are expected to be persistent and bioaccumulative in the environment, based on their similar chemical and physical properties to short-chain chlorinated paraffins (SCCPs).

The analysis of chlorinated paraffins is a challenge. The technical compounds are mixtures of up to 200 congeners with different chain lengths and degrees of chlorination. GC chromatograms of these complex mixtures typically show a lot of overlapping peaks that can be difficult to separate. In particular, the responses to the various chlorination degrees can vary over a large range.

In addition, the presence of sulfochlorinated paraffins and equivalent chain-length chloroalkenes in such technical compounds can cause interference.

This document describes a procedure to compare the chromatogram results for MCCPs compounds from a test sample with the chromatogram results of a defined calibration standard of the most typically used mixture (55 % chlorination for MCCPs). With this GC-ECNI-MS (gas chromatography negative ion chemical ionization mass spectrometry) procedure it uses four ion traces for identifying the MCCPs.

Leather — Determination of chlorinated hydrocarbons in leather —

Part 2:
Chromatographic method for middle-chain chlorinated paraffins (MCCPs)

1 Scope

This document specifies a chromatographic method to determine the amount of middle-chain chlorinated paraffins (MCCPs) C_{14} to C_{17} in processed and unprocessed leathers.

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 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 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

The test sample is extracted using n-hexane at 60 °C in an ultrasonic bath for 60 min. After a sulfuric acid clean-up, an aliquot is then analysed using a gas chromatograph coupled to a mass selective detector with chemical ionization (GC-ECNI-MS).

A liquid chromatography system single quad (LC-MS) or with triple quad mass spectrometry (LC-MS/MS), as described in Annex B, can also be used if the user has demonstrated that the accuracy of measurement is equivalent to that of the GC-ECNI-MS method.

In some cases when determining MCCPs using the GC-ECNI-MS method, the presence of sulfochlorinated paraffins and equivalent chain-length chloroalkenes can cause interference. Annex B proposes a LC-MS/MS application method that aims to give a better resolution and eliminates possible false positives determined with the GC-ECNI-MS method.

5 Apparatus and materials

Use normal laboratory apparatus and, in particular, the following.

5.1 Analytical balance.

5.2 Sealable vessel, with lid, 20 ml, suitable for extraction with n-hexane.

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

NOTE A frequency of 40KHz is suitable.

5.4 Pipette, 1 ml to 10 ml capacity.

5.5 Volumetric flask, 2 ml.

5.6 Gas chromatograph and mass selective detector with chemical ionization (GC-ECNI-MS).

5.7 Shaker or agitator, ensuring an efficient mixing of the phases.

6 Reagents

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

6.1 n-hexane, Chemical Abstracts Service (CAS) No 110-54-3.

6.2 Internal standard (IS) solution, lindane, CAS No 58-89-9, 1 000 µg/ml.

6.3 Standard solutions, MCCPs, C_{14} to C_{17}, with different chlorine content, each 100 µg/ml.

6.3.1 **MCCPs C_{14} to C_{17} 52 % Cl**, technical grade, CAS 85535-85-9.

6.3.2 MCCPs C_{14} to C_{17} 57 % Cl, technical grade.

NOTE These MCCP calibration solutions are available commercially.

6.4 Concentrated sulfuric acid, (ρ = 1,84 g/ml at 20 °C).

7 Sampling

Sample in accordance with ISO 2418. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments), provide details about sampling together with the test report. Glue residuals shall be mechanically removed from leather samples.

Prepare the leather samples according to ISO 4044.

8 Sample preparation and analysis

8.1 Preparation of MCCPs calibration solution (50 µg/ml) with 55 % chlorination degree

Transfer 400 µl MCCPs C_{14} to C_{17} 52 % Cl standard solution (6.3.1) and 600 µl MCCP C_{14} to C_{17} 57 % Cl standard solution (6.3.2) into a 2 ml volumetric flask (5.5). Add 20 µl of internal standard solution (6.2) and fill up the flask to the volume with n-hexane (6.1).

The calibration standard with 55 % chlorine content shall be used for the quantification of all samples, independent of their chlorination degree (if known).

8.2 Extraction of leather

Weigh (0,5 ± 0,001) g test leather sample with the analytical balance (5.1) into the sealable vessel (5.2). Add 9,9 ml n-hexane (6.1) and 100 µl internal standard (6.2) and seal the vessel. Extract the sample at (60 ± 5) °C in an ultrasonic bath (5.3) for (60 ± 2) min. Cool down to room temperature (less than 27 °C).

8.3 Sulfuric acid clean-up

Add a 5 ml aliquot of the extracted hexane solution to a tight sealable vessel (5.2) and add 1 ml sulfuric acid (6.4). Shake the vessel (5.7) for 10 min. After separating of the phases (optionally with centrifugation), 1 ml of the hexane phase shall be taken for analysis.

8.4 GC-ECNI-MS Determination

The solution is analysed using GC-ECNI-MS (5.6). An example of a suitable GC-ECNI-MS method is given in Annex A.

9 Expression of results

9.1 Evaluation

The peak shape evaluation (PSE) have been successfully tested and used. The integration shall be done with PSE, in accordance with examples presented in Annex C.

Peak areas from the four quantification masses of the standard are summed up and equated with standard concentration. Peak areas of the samples are summed up too and the concentration is calculated with the response of the standard.

To check the linearity of the analytical system, a calibration standard as reference standard is analysed after each ten samples and at the end of the sequence. The deviation in reference to the calibration standard should be within ± 20 %, otherwise the analytical system has to be checked before retrying the analysis.

Sample extract should always be diluted in the concentration range of the standard.

The integration of the samples shall only be done in the retention time window of the standard.

9.2 Ions used for quantification

Table A.1 lists the ions used for the quantification of MCCPs and the ions used for the internal standard lindane.

Sum up the quantifier peak areas from the standard and equate with the standard concentration. Also sum up the quantifier peak areas of the sample and calculate the concentration with responses of the calibration standards.

To get a quantitative result, each peak area of the extract chromatogram has to be below the highest calibration point. If not, dilute the extract with IS solution (8.2) in the range or specify that the result is bigger than the calculate result.

9.3 Interference to MCCPs determination

Quantifier m/z (mass-to-charge ratio) ions 431 and 445 shall be quantified only if their area is above 10 % of the quantifier m/z ions 403 or 417 and the masses are not different (peak shape evaluation), see C.3 and Figure C.3.

$C_{17}Cl_7$ quantifier (m/z 445) shows very low response and an overlay with SCCPs ions, therefore areas should be rejected if interference from SCCPs is noticed.

The masses used for MCCPs determination can be interfered with by higher concentrations of SCCPs (>1 %). In this case the MCCPs result in the test report (Clause 10) should be set to “n.d.” with comment: “cannot be determined due to interference from SCCPs in concentrations above 1 %”.

9.4 Calculation

Set up the linear regression function by using the ratio ($A_{\text{MCCP-S}}/A_{\text{int Std}}$) and ($C_{\text{MCCP-S}}/C_{\text{int.Std-S}}$) as shown in Formula (1).

$$\frac{A_{\text{MCCP-S}}}{A_{\text{int. Std-S}}}=\frac{C_{\text{MCCP-S}}}{C_{\text{int.Std-S}}}\cdot a+b \tag{1}$$

where

$A_{\text{MCCP-S}}$	is the sum of the peak areas of MCCPs in the calibration standard;
$A_{\text{int. Std-S}}$	is the area of the internal standard in the calibration standard;
a	is the slope of the linear function;
b	is the intercept of the linear function;
$C_{\text{MCCP-S}}$	is the concentration of MCCPs in the calibration standard, µg/ml;
$C_{\text{int.Std-S}}$	is the concentration of the internal standard in the calibration standard, µg/ml.

The content of the MCCPs in leather is calculated according to Formula (2) as a mass fraction, w, in mg/kg.

$$w=\frac{\left(\frac{A_{\text{MCCP-Sample}}}{A_{\text{int.Std-Sample}}}-b\right)}{a}\cdot\frac{V}{m}\cdot C_{\text{int.Std-Sample}} \tag{2}$$

where

$A_{\text{MCCP-Sample}}$	is the sum of the peak areas of MCCPs in the sample;
$A_{\text{int.Std-Sample}}$	is the peak area of internal standard in the sample;
a	is the slope of the linear function;
b	is the intercept of the linear function;
V	is the final volume, ml;
m	is the mass of the sample, g;
$C_{\text{int .Std-Sample}}$	is the concentration of internal standard in the sample, µg/ml.

9.5 Precision

With this method it is feasible to reach quantification limits of 100 mg/kg.

10 Test report

The test report shall include at least the following:

a) a reference to this document, i.e. ISO 18219-2:2021;

b) all details necessary for complete identification of the sample tested;

c) the date of the test;

d) declaration of analytical techniques used for detection and confirmation;

e) amount of extracted MCCPs (C_{14} to C_{17}) in mg/kg;

f) any deviation by agreement or otherwise from the procedure specified.

Annex A

(informative)

Gas chromatographic (GC-ECNI-MS) analysis operating parameters

A.1 Suggested GC-ECNI-MS conditions

Column:	Non-polar, 5 % phenyl methyl siloxane, e.g. DB-5 or equivalent is suitable length: 25 m internal diameter: 0,25 mm film thickness: 0,25 µm
Carrier gas:	helium, flow rate: 1,2 ml/min
Injector temperature:	250 °C, mode splitless, splitless time: 1,5 min
Injection volume:	1 µl
Temperature programme:	120 °C, up to 300 °C at 12 °C/min, 300 °C for 5 min solvent delay: 4 min total run time: 20 min
MS conditions:	transfer line: 280 °C ion source: 150 °C quadrupole: 120 °C
Chemical ionization (CI) conditions:	CI gas: methane 5,5 (i.e. > 99,9995 % CH_4) CI valve: 40 %

A.2 GC-ECNI-MS system optimization

The system should be optimized for good signal/noise ratio. If the scan-rate is not chosen correctly, a noisy baseline can prevent peak shape interpretation. In this case scan rate should be changed to faster settings, e.g. 2^3.

A.3 Calibration and calculation

Table A.1 — m/z ion signals for quantification and qualification

No.	Substance	Quantifier/qualifier
1	Lindane	325/327 255/253 255/257

Table A.1 *(continued)*

No.	Substance	Quantifier/qualifier
2	MCCPs (C_{14} to C_{17})	403/405 417/419 431/433 445/447

Annex B
(informative)

Liquid chromatographic (LC-MS/MS) analysis operating parameters

B.1 Introduction

A liquid chromatographic system with single quad (LC-MS) or triple quad mass spectrometry (LC-MS/MS) can also be used if the user has demonstrated that the accuracy of measurement is equivalent to that of the GC-ECNI-MS method.

B.2 Apparatus and materials

Use normal laboratory apparatus and, in particular, the following.

B.2.1 Analytical balance, weighing to an accuracy of 0,1 mg.

B.2.2 Sealable vessel, with lid, 20 ml, suitable for extraction with methanol/THF (1:1) mix.

B.2.3 Ultrasonic bath, with controllable heating, capable of maintaining a temperature of (60 ± 5) °C.

NOTE A frequency of 40 KHz is suitable.

B.2.4 Pipette.

B.2.5 Volumetric flask, 2 ml.

B.2.6 Evaporation apparatus, e.g. a rotary vacuum evaporator or a heating block with a controlled flow of gas over the liquid.

B.2.7 High performance liquid chromatograph (HPLC) with triple quad mass selective detectors (LC-MS/MS).

B.2.8 Membrane filter, for example polyamide, 0,45 µm.

B.3 Reagents

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

B.3.1 Methanol, CAS No 67-56-1.

B.3.2 Tetrahydrofuran (THF), CAS No 109-99-9.

B.3.3 n-hexane, CAS No 110-54-3.

B.3.4 Standard solutions, MCCPs, C_{14} to C_{17}, with different chlorine content, each 100 µg/ml.

B.3.4.1 MCCPs C_{14} to C_{17} 52 % Cl, technical grade, CAS No 85535-85-9.

B.3.4.2 MCCPs C_{14} to C_{17} 57 % Cl, technical grade.

NOTE These MCCPs calibration solutions are available commercially.

B.4 Preparation of MCCPs calibration solution (50 µg/ml) with 55 % Cl

Transfer 400 µl MCCPs C_{14} to C_{17} 52 % Cl standard solution (B.3.4.1) and 600 µl MCCPs C_{14} to C_{17} 57 % Cl standard solution (B.3.4.2) into a 2 ml volumetric flask (B.2.5) and fill up to the volume with methanol/THF (1:1) mix. This calibration standard with 55 % chlorine content shall be used for the quantification of all samples, independently of their chlorination degree.

B.5 Extraction of leather and preparation of analytical solution

A (0,5 ± 0,001) g leather sample is weighed with the analytical balance (B.2.1) into the sealable vessel (B.2.2), 10 ml n-hexane (B.3.3) is added and the vessel closed. The sample is extracted for (60 ± 2) min at (60 ± 5) °C in an ultrasonic bath (B.2.3).

The supernatant is recovered and concentrated in the evaporation apparatus (B.2.6) to dryness. The residue is made up to 2 ml with a methanol/THF (1:1) mix. The obtained solution is filtered with a polyamide membrane filter (B.2.8) and placed into the vial for the HPLC analysis (B.2.7).

B.6 Instrumental parameters

LC-MS conditions

Column:	reverse phase C18 column with TMS capping, 2,6 µm, 100 × 2,1 mm
Eluent A:	water
Eluent B:	methanol
Column oven:	35 °C
Gradient programme:	as reported in Table B.1.

Table B.1 — Gradient programme

Time min	% A	% B
0.01	40	60
15.00	0	100
21.00	0	100
22.00	40	60
30.00	stop	stop

Flow rate:	0,2 ml/min
Injection Volume:	2,0 µl
Scan (-):	80 Da to 1000 Da
Selected ion monitoring (SIM):	The result is evaluated as the total area of SIM signals reported in Table B.2.

Table B.2 — m/z ions for MCCPs (STD C_{14} to C_{17})

$C_{14}H_{22}Cl_8$(-)	473 to 475
$C_{14}H_{21}Cl_9$(-)	507 to 509
$C_{14}H_{18}Cl_{12}$(-)	609 to 611
$C_{14}H_{13}Cl_{17}$(-)	785 to 783 to 787
$C_{15}H_{24}Cl_8$(-)	487 to 489
$C_{15}H_{21}Cl_{11}$(-)	589 to 587
$C_{15}H_{18}Cl_{14}$(-)	693 to 695
$C_{15}H_{15}Cl_{17}$(-)	797 to 801
$C_{16}H_{27}Cl_7$(-)	467 to 469 to 471
$C_{16}H_{25}Cl_9$(-)	535 to 537
$C_{16}H_{20}Cl_{14}$(-)	705 to 707
$C_{17}H_{28}Cl_8$(-)	513 to 515
$C_{17}H_{27}Cl_9$(-)	549 to 547 to 551
$C_{17}H_{22}Cl_{14}$(-)	719 to 721
$C_{17}H_{21}Cl_{15}$(-)	753 to 755

To get a quantitative result, each peak area of the extract chromatogram has to be below the highest calibration point. If not, dilute the extract with IS solution (8.1) in the range or specify the result is bigger than the calculated result.

An example of the chromatogram of the MCCPs standard (C_{14} to C_{17}, 52 % Cl) using LC-MS/MS is shown in Figure B.1.

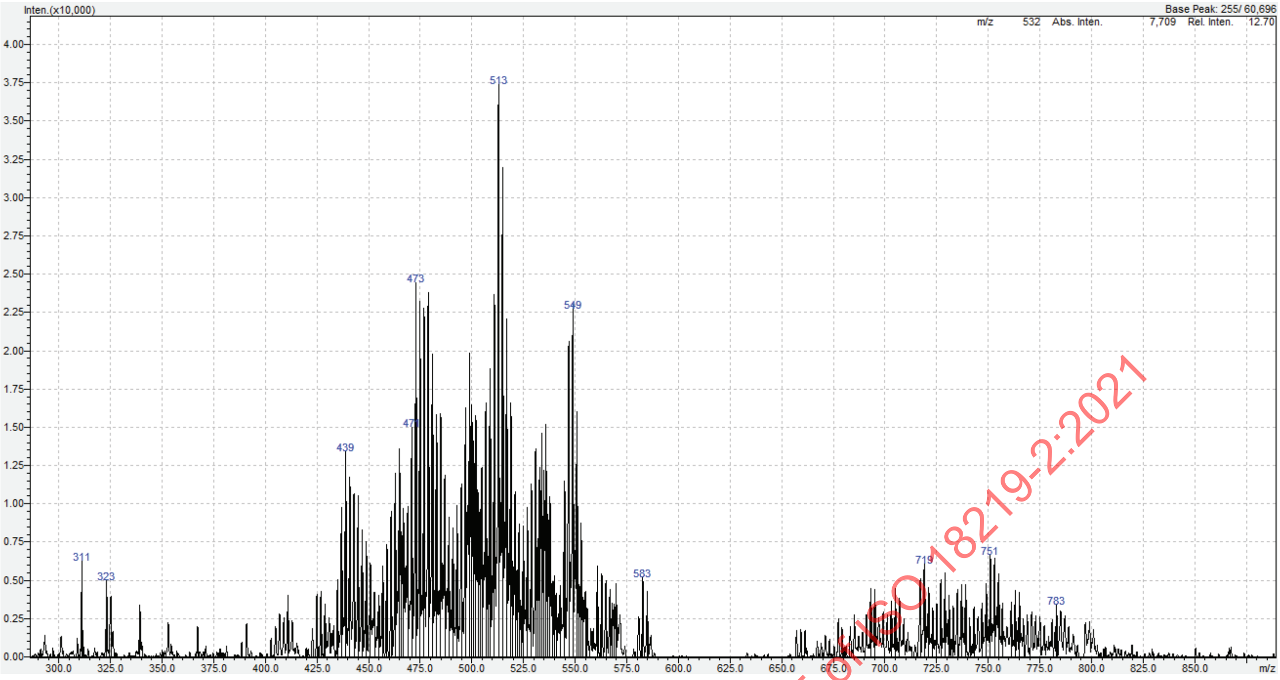

Figure B.1 — Example of chromatogram of MCCPs analysis with LC-MS/MS

B.7 Calculation

The content of the MCCPs in leather is calculated according to the calibration obtained from MCCPs calibration solution (B.1) and ranging from 2,5 µg/ml to 20 µg/ml.

The result is calculated as a mass fraction, w, in mg/kg according to Formula (B.1):

$$w = \frac{C_{\text{MCCP}} \cdot V}{m_{\text{s}}} \qquad \text{(B.1)}$$

where

C_{MCCP} is the concentration of MCCPs obtained from calibration curve, µg/ml;

V is the final volume, ml;

m_{s} is the mass of the sample, g.