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Leather — Chemical tests — Determination of certain azo colourants in dyed leathers

Cuir — Essais chimiques — Dosage de certains colorants azoïques dans les cuirs teints

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 17234 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). This method is technically similar to the method in IUC 20 which was declared an official method at the IULTCS Delegates meeting on 31st May 2003 in Cancun, Mexico. This edition differs slightly in the text compared with IUC 20.

Leather — Chemical tests — Determination of certain azo colourants in dyed leathers

1 Scope

This Technical Specification specifies a method for determining the use of certain azo colourants which may release certain aromatic amines.

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 2418:2002, *Leather – Chemical, physical and mechanical and fastness tests – Sampling location*

ISO 3696:1987, *Water for analytical laboratory use – Specification and test methods*

ISO 4044:1977, *Leather – Preparation of chemical test samples*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
certain azo colourants
certain azo colourants are such colourants as may, by cleavage of their azo group or azo groups, form the amines listed below

According to the current state of scientific knowledge, evidence of the use of banned azo colourants in the manufacture or treatment of leathers is said to exist, where the coloured leather yields upon cleavage under the conditions of this procedure (8.2) one of the following amines and the determined level of any of these amines exceeds 30 mg/kg.

1)	4-Aminodiphenyl	(CAS-No.: 92-67-1)
2)	Benzidine	(CAS-No.:92-87-5)
3)	4-Chlorotoluidine	(CAS-No.: 95-69-2)
4)	2-Naphthylamine	(CAS-No.: 91-59-8)
5)	4-Chloroaniline	(CAS-No.: 106-47-8)
6)	2,4-Diaminoanisole	(CAS-No.: 615-05-4)
7)	4,4'-Diaminodiphenylmethane	(CAS-No.: 101-77-9)

8)	3,3'-Dichlorobenzidine	(CAS-No.: 91-94-1)
9)	3,3'-Dimethoxybenzidine	(CAS-No.: 119-90-4)
10)	3,3'-Dimethylbenzidine	(CAS-No.: 119-93-7)
11)	3,3'-Dimethyl-4,4'-diaminodiphenylmethane	(CAS-No.: 838-88-0)
12)	4-Cresidine	(CAS-No.: 120-71-8)
13)	4,4'-Methylene-bis-(2-chloroaniline)	(CAS-No.: 101-14-4)
14)	4,4'-Oxydianiline	(CAS-No.: 101-80-4)
15)	4,4'-Thiodianiline	(CAS-No.: 139-65-1)
16)	2-Toluidine	(CAS-No.: 95-53-4)
17)	2,4-Diaminotoluene	(CAS-No.: 95-80-7)
18)	2,4,5-Trimethylaniline	(CAS-No.: 137-17-7)

Note CAS = Chemical Abstract Service

According to this method, azo colourants which yield upon cleavage of their azo groups the amines 2-aminoazotoluene (CAS-No.: 97-56-3) and 2-amino-4-nitrotoluene (CAS-No.: 99-55-8), are detected via the amines 2-toluidine and/or 2,4-diaminotoluene.

4 Principle

After degreasing, the leather sample is treated with sodium dithionite in an aqueous buffer solution (pH 6) at 70 °C in a closed vessel. The amines released in the process of reductive cleavage are transferred to a *t*-butyl methyl ether phase by means of liquid-liquid extraction using kieselgur columns. The *t*-butyl methyl ether extract is then concentrated under mild conditions in a rotary vacuum evaporator and the residue is dissolved in a suitable solvent, depending on the method used to determine the amines.

Determination of the amines is performed by means of high pressure liquid chromatography using a diode array detector (HPLC/DAD), thin layer chromatography (TLC, HPTLC) and densitometric quantification, capillary gas chromatography with a flame ionisation detector and/or a mass specific detector (GC/FID and/or MSD), or by capillary electrophoresis with a diode array detector (CE/DAD).

The amines must be identified by means of at least two different chromatographic separation methods in order to avoid any possible misinterpretations caused by interfering substances (such as position isomers of the amines to be identified) and hence any incorrect statements. Amine quantification is performed by HPLC/DAD.

5 Special equipment and supplies

Usual laboratory equipment and in particular the following:

5.1 Suitable reaction vessel of temperature-resistant glass with gas tight closure.

5.2 Hot cabinet with sand bath (sea sand 0,1-0,3 mm) or water bath with thermostat

5.3 Thermometer, 0,5 °C accuracy at 70 °C

5.4 Volumetric flasks, different volumes

5.5 Polypropylene or glass column with 25 mm to 30 mm inner diameter and 140 mm to 150 mm length, glass filter at the outlet, filled with porous granulated kieselguhr [e.g. Extralut NT20, prefilled columns (Merck Art. No. 11737)].

5.6 Polypropylene or polyethylene syringe, 2 ml

5.7 Vacuum rotary evaporator

5.8 Pipettes, 10 ml, 5 ml, 2 ml, 1 ml

5.9 Ultrasonic bath with thermostat

5.10 100 ml round-bottomed flask with standard ground joint NS 29132

5.11 Instrumental analysis

- automatic applicator for HPTLC or TLC
- Densitometer
- Capillary electrophoresis with DAD
- Capillary GC, split/splitless injector, preferably with MS/MSD
- HPLC with gradient controller, preferably with DAD, or HPLC-MS

6 Chemicals

Unless otherwise specified, analytical grade chemicals have to be used.

6.1 Methanol

6.2 *t*-Butyl methyl ether

6.3 Sodium dithionite, minimum 87 % purity

6.4 Aqueous sodium dithionite solution, 200 mg/ml, prepared daily

6.5 n-Hexane

6.6 Amines 1 to 18 according to clause 3.1 (highest available purity standard)

6.7 Stock solution of the amines (6.6) : 400 mg/l in ethyl acetate for TLC

6.8 Stock solution of the amines (6.6) : 200 mg/l in methanol for GC, HPLC, CE

6.9 Citrate buffer solution, 0,06 M, pH 6 (e.g. Merck Art. No. 1.09437.1 000), preheated to 70 °C

6.10 Standard solution for amine process control: 30 µg amine /ml solvent, freshly prepared from stock solutions (6.7) or (6.8) depending on the analytical method

6.11 20 % (w/v) methanolic NaOH, 20 g NaOH dissolved in 100 ml methanol

6.12 Water of Grade 3 according to ISO 3696.

7 Sampling and preparation of samples

If possible sample in accordance with ISO 2418 and grind the leather in accordance with ISO 4044:1977. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments) details about sampling have to be given in the test report.

For the analytical procedure, a representative sample of 1,0 g of this ground leather is weighed accurately into the reaction vessel (5.1).

Remove traces of adhesives where necessary mechanically.

8 Procedure

8.1 Degreasing

1 g of the ground leather sample is treated in a closed 50 ml vessel (5.1) with 20 ml hexane (6.5) in an ultrasonic bath (5.9) at 40 °C for 20 minutes. The hexane layer is decanted from the leather sample. Directly afterwards the sample is treated again in the same way as before with 20 ml hexane. The residual hexane is evaporated overnight in the open vessel.

8.2 Reductive cleavage

A quantity of 17 ml buffer solution (6.9) preheated to 70 °C ± 5 °C is added to the sample. The reaction vessel (5.1) is tightly sealed, and, after shaking, kept in a ventilated oven in a sand bath or in a heatable bath (5.2) for 25 ± 5 min at 70 °C ± 2 °C. The reaction temperature of 70 °C has to be reached inside the reaction vessel. This has to be checked with an additional vessel with thermometer inside.

Then 1,5 ml aqueous sodium dithionite solution (6.4) are added with a syringe (5.6) and the vessel is kept for 10 min at 70 °C. Afterwards another 1,5 ml sodium dithionite solution is added and the vessel is heated for another 10 min and cooled to room temperature with water.

8.3 Liquid liquid extraction

Using a glass pestle, the reaction solution is squeezed out of the fibres, decanted on the Kieselguhr column (5.5) and allowed to be absorbed by the column for 15 min.

5 ml of *t*-butyl methyl ether (6.2) and 1 ml of 20 % methanolic NaOH (6.11) are added to the leather fibre residue in the vessel. The vessel is closed and shaken vigorously and transferred to the Kieselguhr column (5.5).

Wash the reaction vessel and fibre residues with 1 × 15 ml and 1 × 20 ml *t*-butyl methyl ether and transfer to the Kieselguhr column to begin eluting the amines. Afterwards 40 ml t-butyl methyl ether are flushed directly on the column. The eluate is collected in a 100 ml round-bottomed flask with standard ground joint (5.10).

The *t*-butyl methyl ether extract is concentrated to approx. 1 ml (not to dryness!) in a rotary vacuum evaporator (5.7) in a slight vacuum at not more than 50 °C. The remainder of the ether is then evaporated to dryness using a slight flow of inert gas.

The residue is immediately transferred to a 2 ml volumetric flask (5.4) and made up to volume with methanol (or ethyl acetate for TLC analytical method). This solution is ready for the instrumental analysis.

8.4 Check of the analytical system

To check the analysis procedure, 1,0 ml of the standard solution (6.10) is added to a reaction vessel (5.1) containing 16 ml of the preheated buffer (6.9). Then the procedure set out in chapter 8.2 and following is carried out. The recovery rate shall be at least 70 % for amines 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, at least 50 % for amines 16, 17 and 20 % for Amine 6.

9 Calibration

The standard solution (6.10) with 30 µg/ml is used for calibration.

10 Chromatographic analyses

As various types of equipment may be used, general statements cannot be made. The following parameters have been successfully tested and used in these analyses.

10.1 Chromatographic analyses for quantitative and qualitative detection: High Performance Liquid Chromatography (HPLC)

Eluent 1:	Methanol;
Eluent 2:	0,575 g Ammonium dihydrogen phosphate + 0,7 g disodium hydrogen phosphate in 1 000 ml water, pH 6,9;
Stationary phase:	LiChrospher 60 RP-select B (5µm) 250 mm x 4,6 mm;
Column temperature:	40 °C
Flow rate:	0,8 - 1,0 ml/min;
Gradient:	Start 15 % eluent 1, linear increase to 80 % eluent 1 within 45 minutes;
Injection volume:	10 µl;
Detection:	DAD at 240 nm, 280 nm and 305 nm.

10.2 Chromatographic analyses for qualitative detection

10.2.1 Capillary Gas Chromatography (GC)

Capillary column:	medium polarity, e.g. SE 54 or DB 5, length: 50 m, inner diameter: 0,32 mm, film thickness: 0,5 mm
Injection system:	split/splitless
Injector temperature:	250 °C
Temperature Programme:	70 °C for 2 minutes, up to 280 °C at 10 °C / min, 280 °C for 5 minutes;
Detector:	MSD, scan 45-300 amu
Carrier gas:	Helium
Injection:	1 µl, splitless 2 minutes