INTERNATIONAL STANDARD

ISO 17234-1

IULTCS/IUC 20-1

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Leather — Chemical tests for the determination of certain azo colorants in dyed leathers —

Part 1: Determination of certain aromatic amines derived from azo colorants

Cuir — Essais chimiques pour le dosage de certains colorants azoïques dans les cuirs teints —

Partie 1: Dosage de certaines amines aromatiques dérivées des colorants azoïques

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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.

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 17234-1 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 289, *Leather*, in collaboration with the Chemical Test Commission of the International Union of Leather Technologists and Chemists Societies (IUC Commission, IULTCS), 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.

IULTCS, originally formed in 1897, is a worldwide 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 17234-1 cancels and replaces ISO/TS 17234:2003, which has been technically revised.

ISO 17234 consists of the following parts, under the general title *Leather — Chemical tests for the determination of certain azo colorants in dyed leathers*:

— *Part 1: Determination of certain aromatic amines derived from azo colorants*

— *Part 2: Determination of 4-aminoazobenzene*

Leather — Chemical tests for the determination of certain azo colorants in dyed leathers —

Part 1:
Determination of certain aromatic amines derived from azo colorants

1 Scope

This part of ISO 17234 specifies a method for determining the use of certain azo colorants 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, *Leather — Chemical, physical and mechanical and fastness tests — Sampling location*

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

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

ISO 17234-2, *Leather — Chemical tests for the determination of certain azo colorants in dyed leathers — Part 2: Determination of 4-aminoazobenzene*

3 General

Certain azo colorants may release, by reductive cleavage of azo group(s), one or more of the following aromatic amines, which are listed in Appendix 8 of EU regulation 1907/2006 (see Table 1).

According to the current state of scientific knowledge, the use of banned azo colorants in the manufacture or treatment of leathers is considered as proved if the coloured leather yields upon cleavage, under the conditions of this procedure (see 9.2), one or more of the amines indicated in Table 1 and the determined amount of any of these exceeds 30 mg/kg.

Table 1 — Aromatic amines listed in Appendix 8 of EU regulation 1907/2006

No.	CAS number	Index number	EC number	Substances
1	92-67-1	612-072-00-6	202-177-1	biphenyl-4-ylamine 4-aminobiphenyl xenylamine
2	92-87-5	612-042-00-2	202-199-1	benzidine
3	95-69-2		202-441-6	4-chloro-*o*-toluidine
4	91-59-8	612-022-00-3	202-080-4	2-naphthylamine
5[a]	97-56-3	611-006-00-3	202-591-2	*o*-aminoazotoluene 4-amino-2',3-dimethylazobenzene 4-*o*-tolylazo-*o*-toluidine
6[a]	99-55-8		202-765-8	5-nitro-*o*-toluidine
7	106-47-8	612-137-00-9	203-401-0	4-chloroaniline
8	615-05-4		210-406-1	4-methoxy-*m*-phenylenediamine
9	101-77-9	612-051-00-1	202-974-4	4,4'-methylenedianiline 4,4'-diaminodiphenylmethane
10	91-94-1	612-068-00-4	202-109-0	3,3'-dichlorobenzidine 3,3'-dichlorobiphenyl-4,4'-ylenediamine
11	119-90-4	612-036-00-X	204-355-4	3,3'-dimethoxybenzidine *o*-dianisidine
12	119-93-7	612-041-00-7	204-358-0	3,3'-dimethylbenzidine 4,4'-bi-*o*-toluidine
13	838-88-0	612-085-00-7	212-658-8	4,4'-methylenedi-*o*-toluidine
14	120-71-8		204-419-1	6-methoxy-*m*-toluidine *p*-cresidine
15	101-14-4	612-078-00-9	202-918-9	4,4'-methylene-bis-(2-chloro-aniline) 2,2'-dichloro-4,4'-methylene-dianiline
16	101-80-4		202-977-0	4,4'-oxydianiline
17	139-65-1		205-370-9	4,4'-thiodianiline
18	95-53-4	612-091-00-X	202-429-0	*o*-toluidine 2-aminotoluene
19	95-80-7	612-099-00-3	202-453-1	4-methyl-*m*-phenylenediamine
20	137-17-7		205-282-0	2,4,5-trimethylaniline
21	90-04-0	612-035-00-4	201-963-1	*o*-anisidine 2-methoxyaniline
22[b]	60-09-3	611-008-00-4	200-453-6	4-aminoazobenzene

[a] The CAS numbers 97-56-3 (No. 5) and 99-55-8 (No. 6) are further reduced to CAS numbers 95-53-4 (No. 18) and 95-80-7 (No. 19).

[b] Azo colorants that are able to form 4-aminoazobenzene generate under the condition of this method aniline and/or 1,4-phenylenediamine. The presence of these colorants shall be tested using ISO 17234-2.

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 shall 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 shall be performed by HPLC/DAD.

5 Safety precautions

5.1 Aromatic amines listed in Clause 3 are classified as substances known to be or suspected to be human carcinogens.

Any handling and disposal of these substances shall be strictly in accordance with the appropriate national health and safety regulations.

5.2 It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Consult manufacturers for specific details such as material safety data sheets and other recommendations.

5.3 Good laboratory practice should be followed. Wear safety glasses in all laboratory areas and a single-use dust respirator and single-use gloves while handling powder colorants and aromatic amines.

5.4 Users shall comply with any national and local safety regulations.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 **Suitable reaction vessel**, of temperature-resistant glass with gas-tight closure.

6.2 **Hot cabinet with sand bath** (sea sand, 0,1 mm to 0,3 mm) or **water bath** with thermostat.

6.3 **Thermometer**, 0,5 °C accuracy at 70 °C.

6.4 **Volumetric flasks**, different volumes.

6.5 **Polypropylene** or **glass column**[1)], of 25 mm to 30 mm inner diameter and of 140 mm to 150 mm length, with a glass filter at the outlet and filled with porous granulated Kieselgur.

6.6 **Polypropylene** or **polyethylene syringe**, 2 ml.

6.7 **Vacuum rotary evaporator**.

6.8 **Pipettes**, 10 ml, 5 ml, 2 ml, 1 ml.

1) The EXtrelut® NT20 prefilled column supplied by Merck is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 17234 and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

6.9 Ultrasonic bath with thermostat.

6.10 Round-bottomed flask, of 100 ml with standard ground joint NS 29132.

6.11 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.

7 Reagents

Unless otherwise specified, use analytical grade chemicals.

7.1 Methanol.

7.2 *t*-Butyl methyl ether.

7.3 Sodium dithionite, minimum 87 % purity.

7.4 Aqueous sodium dithionite solution, 200 mg/ml, prepared daily.

7.5 *n*-Hexane.

7.6 Amines, listed in Table 1 (highest available purity standard).

7.7 Stock solution of the amines (7.6): 400 mg/l in ethyl acetate for TLC.

7.8 Stock solution of the amines (7.6): 200 mg/l in methanol for GC, HPLC, CE.

7.9 Citrate buffer solution[2)], 0,06 mol/l, pH 6, preheated to (70 ± 5) °C.

7.10 Standard solution for amine process control, 30 µg amine per millilitre solvent, freshly prepared from stock solutions (7.7) or (7.8) depending on the analytical method.

7.11 20 % **methanolic NaOH solution**, 20 g NaOH dissolved in 100 ml methanol.

7.12 Water, Grade 3 according to ISO 3696:1987.

8 Sampling and preparation of samples

Sample in accordance with ISO 2418 and grind the leather in accordance with ISO 4044. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments, etc.), details about sampling shall be given in the test report. Any traces of adhesives shall be removed mechanically.

For the analytical procedure, accurately weigh a representative sample of 1,0 g of this ground leather in the reaction vessel (6.1).

2) The solution No. 1.09437.1 000 supplied by Merck is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 17234 and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

9 Procedure

9.1 Degreasing

Treat 1 g of the ground leather sample in a closed 50 ml vessel (6.1) with 20 ml *n*-hexane (7.5) in an ultrasonic bath (6.9) at 40 °C for 20 min. Decant the *n*-hexane layer from the leather sample. Any loss of leather particles during decanting shall be avoided. Directly after decanting, treat the sample again in the same way as before with 20 ml *n*-hexane. Evaporate the residual *n*-hexane overnight in the open vessel.

9.2 Reductive cleavage

Add a quantity of 17 ml buffer solution (7.9) preheated to (70 ± 5) °C to the sample. Tightly seal the reaction vessel (6.1), shake it, and keep it in a ventilated oven, in a sand bath or in a heatable bath (6.2) for (25 ± 5) min at (70 ± 2) °C. The reaction temperature of 70 °C shall be reached inside the reaction vessel. This shall be checked with an additional vessel with a thermometer inside.

Add 1,5 ml aqueous sodium dithionite solution (7.4) with a syringe (6.6) and keep the vessel at 70 °C for 10 min. Afterwards, add another 1,5 ml sodium dithionite solution and heat the vessel for another 10 min. Then cool it to room temperature with water.

9.3 Liquid-liquid extraction

Using a glass pestle, squeeze the reaction solution out of the fibres, decant on the Kieselgur column (6.5) and allow absorption by the column for 15 min.

Add 5 ml of *t*-butyl methyl ether (7.2) and 1 ml of 20 % methanolic NaOH (7.11) to the leather fibre residue in the vessel. Close the vessel, shake it vigorously and transfer the solution to the Kieselgur column (6.5).

Wash the reaction vessel and fibre residues with 1 × 15 ml and 1 × 20 ml *t*-butyl methyl ether and transfer to the Kieselgur column to begin eluting the amines. Afterwards, directly flush 40 ml *t*-butyl methyl ether on the column. Collect the eluate in a 100 ml round-bottomed flask with standard ground joint (6.10).

Concentrate the *t*-butyl methyl ether extract to approximately 1 ml (not to dryness) in a rotary vacuum evaporator (6.7) in a slight vacuum at not more than 50 °C. Then evaporate the remainder of the ether to dryness using a slight flow of inert gas.

Immediately transfer the residue to a 2 ml volumetric flask (6.4) and make up to volume with methanol (or ethyl acetate for TLC analytical method). This solution is ready for the instrumental analysis.

9.4 Check of the analytical system

To check the analysis procedure, add 1,0 ml of the standard solution (7.10) to a reaction vessel (6.1) containing 16 ml of the preheated buffer solution (7.9). Then carry out the procedure described in 9.2 and 9.3. Amine recovery rates shall comply with the following minimum requirements:

— amines Nos. 1 to 4, 7, 9 to 17, 20 and 21: recovery rate 70 %;

— amine No. 8: recovery rate 20 %;

— amines Nos. 18 and 19: recovery rate 50 %;

— amines Nos. 5, 6 and 22, see footnotes to Table 1.

10 Calibration

Use the standard solution (7.10) containing 30 µg amine/ml for calibration.

11 Chromatographic analyses

11.1 General

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.

11.2 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[3)] (5 µm) 250 mm × 4,6 mm;
Column temperature:	40 °C;
Flow rate:	0,8 ml/min to 1,0 ml/min;
Gradient:	start: 15 % eluent 1, then linear increase to 80 % eluent 1 within 45 min;
Injection volume:	10 µl;
Detection:	DAD at 240 nm, 280 nm and 305 nm.

11.3 Chromatographic analyses for qualitative detection

11.3.1 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 min, up to 280 °C at 10 °C/min, 280 °C for 5 min;
Detector:	MSD, scan 45-300 amu;
Carrier gas:	helium;
Injection:	1 µl, splitless, 2 min.

11.3.2 Capillary electrophoresis (HPCE)

Mix 250 µl of the sample solution with 50 µl HCl (c = 0,01 mol/l) and pass through a membrane filter (0,2 µm). Analyse this solution by means of capillary zone electrophoresis.

Capillary 1:	56 cm, uncoated, inside diameter: 50 µm, with extended light path;

3) LiChrospher 60 RP-select B is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 17234 and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

Capillary 2: 56 cm, coated with polyvinyl alcohol (PVA), inside diameter: 50 µm, with extended light path;

Buffer solution: phosphate buffer solution (c = 50 mmol/l), pH 2,5;

Column temperature: 25 °C;

Voltage: 30 kV;

Injection time: 4 s;

Flushing time: 5 s;

Detection: DAD 214 nm, 240 nm, 280 nm, 305 nm.

11.3.3 Thin layer chromatography (TLC)

11.3.3.1 Plates (HPTLC): silica gel with fluorescence indicator F 254, 20 cm × 10 cm;

Applied volume: 5 µl applied as line with automatic applicator;

Mobile solvent: chloroform:acetic acid 90:10 parts by volume;

11.3.3.2 Plates (TLC): silica gel 60, 20 × 10 cm, saturated chamber;

Applied volume: 10 µl applied as a dot with an automatic applicator;

Mobile solvent 1: chloroform:ethyl acetate:acetic acid = 60:30:10 parts by volume;

Mobile solvent 2: chloroform:methanol = 95:5 parts by volume;

Reagent 1: 0,1 % $NaNO_2$ in KOH (c = 1 mol/l);

Reagent 2: 0,2 % alpha-naphthol, in KOH (c = 1 mol/l).

12 Evaluation

Calculate the amine concentration based on the peak areas of the individual amine components with reference to the 30 µg/ml calibration group of amines (7.10). Calculate the amine content as mass portions, w, in milligrams of the individual component per kilogram of leather material according to the following equation:

$$w = \frac{A_P \cdot \rho_K \cdot V}{A_K \cdot m}$$

where

A_P is the peak area of the amine, in area units, in the sample;

A_K is the peak area of the amine, in area units, in the calibration solution;

ρ_K is the concentration of the amine in the calibration solution, in micrograms per millilitre;

V is the volume to which the sample is made up in 9.3 (final sample volume), in millilitres;

m is the mass portion accounted for by the leather sample in the final sample volume, in grams.

13 Analysis report

Any analysis report shall refer to this analytical procedure and state the following details:

a) a reference to this part of ISO 17234;

b) type, origin and designation of the analysed article or part thereof;

c) any deviations from the analytical procedure, particularly any additional steps performed;

d) declaration of the performed separation procedure and methods used for detection and determination (a second method shall be used for confirmation of a positive result);

e) the analytical results for the amines, in milligrams per kilogram (see Clause 12), shall be individually listed and reported according to the identification threshold values as follows:

In the case of levels per amine component < 30 mg/kg:

According to the analysis as carried out, azo colorants which release the listed aromatic amines were not detected.

In the case of levels per amine component > 30 mg/kg:

The analysis result suggests that the leather submitted has been manufactured or treated using azo colorants which release one or more of the listed amines.

In the case of levels of 4-aminodiphenyl and/or 2-naphthylamine > 30 mg/kg:

Use of this analytical method has detected 4-aminodiphenyl and/or 2-naphthylamine. According to the current state of knowledge, it can not be unequivocally confirmed without additional information that azo colorants which release amines were used.

14 Precision of the method

The data indicated in Table 2 were obtained in an interlaboratory collaborative trial on different kinds of leathers. The data were obtained by using HPLC with DAD. The samples were ground according to ISO 4044. For liquid-liquid extraction, Merck columns, type EXtrelut® NT20[1)] Part No. 11737, were used.

Table 2 — Interlaboratory trial — Precision data

Leather sample	Detected amines	Mean mg/kg	Repeatability mg/kg r	Reproducibility mg/kg R
A	Benzidine	13,5	5,4	8,4
	3,3'-Dimethoxybenzidine	15,4	4,4	6,4
	3,3'-Dimethylbenzidine	20,5	7,1	9,5
B	Benzidine	12,9	3,8	8,9
	2-Toluidine	37,5	15,4	38,5
C	3,3'-Dimethylbenzidine	25,6	8,0	17,0
	2-Toluidine	50,1	20,2	42,1
D	Benzidine	16,5	3,0	7,1

Bibliography

[1] Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006, *Official Journal of the European Union*, L136, 29.5.2007. Available at the address (2009-09-25): http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:136:0003:0280:en:PDF

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