International Standard

ISO 17234-1

IULTCS IUC 20-1

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

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

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.

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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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.

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).

It is based on IUC 20 published in J. Soc. Leather Tech. Chem., **86**, pp. 299-305, 2002, and declared an official method of the IULTCS in June 2003.

This fourth edition cancels and replaces the third edition (ISO 17234-1:2020), which has been technically revised.

The main changes are as follows:

— normative Annexes E and F have been added.

A list of all parts in the ISO 17234 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.

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

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

1 Scope

This document specifies a method to determine certain aromatic amines derived from azo colourants.

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*

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

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 General

Certain azo colourants can release, by reductive cleavage of azo group(s), one or more of the aromatic amines listed in EU Regulation 1907/2006, Annex XVII, Appendix 8[2] and GB 20400-2006[3] (see Table 1).

Table 1 — Aromatic amines listed in EU Regulation 1907/2006, Annex XVII, Appendix 8[2] and GB 20400-2006[3]

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	612–196–00–0	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	612–210–00–5	202–765–8	5-nitro-*o*-toluidine 2-amino-4-nitrotoluene
7	106–47–8	612–137–00–9	203–401–0	4-chloroaniline
8	615–05–4	612–200–00–0	210–406–1	4-methoxy-*m*-phenylenediamine 2,4-diaminoanisole
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	612–209–00-X	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	612–199–00–7	202–977–0	4,4'-oxydianiline
17	139–65–1	612–198–00–1	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 2,4-toluylendiamine 2,4-diaminotoluene
20	137–17–7	612–197–00–6	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
23[c]	95–68–1	612–027–00–0	202–440–0	2,4-xylidine 2,4-dimethylbenzene-1-amine
24[c]	87–62–7	612–161–00-X	201–758–7	2,6-xylidine 2,6-dimethylbenzene-1-amine

[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 colourants that are able to form 4-aminoazobenzene generate under the condition of this method aniline (CAS-number 62–53–3) and 1,4-phenylenediamine (CAS number 106–50–3). The presence of these colourants shall be tested using ISO 17234-2.

[c] Additional aromatic amines in GB 20400–2006.

5 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 (8.5) phase by means of liquid-liquid extraction using diatomaceous earth columns. The *t*-butyl methyl ether (8.5) 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 (see Annex A).

Determination of the amines is performed by means of liquid chromatography (LC) using a diode array detector (DAD) or mass selective detector (LC-MS), by capillary gas chromatography with a mass selective detector (GC-MS) or by capillary electrophoresis with a diode array detector (CE-DAD), or qualitatively with (high-performance) thin layer chromatography (TLC, HPTLC).

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 LC-DAD, LC-MS or GC-MS.

A screening method using liquid-liquid extraction without diatomaceous earth columns is described in Annex D.

If it is required to analyse the colourant itself, the method in Annex E shall be used.

If it is required to analyse for residual free aromatic amines in the leather or colourant, the method in Annex F shall be used.

6 Safety precautions

WARNING — The aromatic amines listed in Clause 4 are classified as substances known to be or suspected to be human carcinogens.

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

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

6.3 National and local safety regulations can apply.

7 Apparatus

The usual laboratory equipment and, in particular, the following is used.

7.1 **Suitable reaction vessel**, made of temperature-resistant glass with a gas-tight closure.

7.2 **Suitable heating system,** at (70 ± 2) °C.

7.3 **Polypropylene** or **glass column**, inside diameter 25 mm to 30 mm, length 130 mm to 150 mm, packed with 20 g of diatomaceous earth, fitted with glass fibre filter at the outlet.

The diatomaceous earth columns are either bought pre-packed and used as is, or 20 g of diatomaceous earth can be packed into a glass or polypropylene column of the dimensions given.

7.4 **Vacuum rotary evaporator with vacuum control and water bath**.

7.5 Pipettes, in required sizes or variables pipettes.

7.6 Ultrasonic bath with thermostat.

7.7 Chromatographic equipment, selected from the following.

7.7.1 Liquid chromatography (LC) and DAD or MS.

7.7.2 Capillary gas chromatography (GC), with MS.

7.7.3 Capillary electrophoresis (CE), with DAD.

7.7.4 Thin layer chromatography (TLC) or **high-performance thin layer chromatography (HPTLC)**.

NOTE A description of the chromatographic equipment (7.7) is given in Annex A.

8 Reagents

Unless otherwise specified, analytical grade chemicals shall be used.

8.1 *n*-hexane.

8.2 Citrate buffer solution, 0,06 mol/l, pH = 6, preheated to (70 ± 2) °C.

8.3 Aqueous sodium dithionite solution, ρ = 200 mg/ml[1)], freshly prepared, to be used immediately after resting for 1 h in a closed vessel.

8.4 Sodium hydroxide aqueous solution, a mass fraction of 40 %.

8.5 *t*-butyl methyl ether.

8.6 Methanol.

8.7 Acetonitrile.

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

8.9 Standard solutions.

8.9.1 Stock solution of the amines (8.8), 400 µg/ml in ethyl acetate for TLC.

8.9.2 Stock solution of the amines (8.8), 200 µg/ml of each amine in an appropriate solvent.

NOTE Acetonitrile is an appropriate solvent for this stock solution, resulting in good stability of amines.

8.9.3 Standard solution for amine process control, 30 µg amine per millilitre solvent, freshly prepared from stock solutions (8.9.1 or 8.9.2) depending on the analytical method.

1) ρ = mass concentration.

8.9.4 Internal standard in solution (IS), ρ = 10 µg of IS/ml of *t*-butyl methyl ether (8.5).

In the case of GC-MS analysis, one of the following internal standards can be used:

— IS1: naphthalene-d8, CAS no. 1146-65-2;

— IS2: 2,4,5-trichloroaniline (TCA), CAS no. 636-30-6;

— IS3: anthracene-d10, CAS no. 1719-06-8.

8.10 Water, Grade 3 according to ISO 3696.

9 Sampling and preparation of samples

The leather shall be sampled in accordance with ISO 2418 and prepared in accordance with ISO 4044. If sampling in accordance with ISO 2418 is not possible (e.g. in the case of leathers from finished products such as shoes or garments), details about sampling shall be given in the test report. Any traces of adhesives shall be removed mechanically.

In the case of leather patchwork fabrics with varicoloured patterns, the various colours shall be taken into account separately as far as possible. For commodities consisting of various leather qualities, specimens of the various qualities shall be analysed separately.

10 Procedure

10.1 Degreasing

Weigh a representative specimen of (1,0 ± 0,1) g to the nearest 0,01 g of the leather sample in the reaction vessel (7.1), and add 40 ml *n*-hexane (8.1). Close the vessel (7.1) and put it in an ultrasonic bath (7.6) at (40 ± 2) °C for (40 ± 5) min.

Decant the *n*-hexane layer from the leather specimen. Any loss of leather particles during decanting shall be avoided. Evaporate the residual *n*-hexane at least overnight in the open vessel.

10.2 Reductive cleavage

Add 15 ml buffer solution (8.9) preheated to (70 ± 2) °C to the sample.

Close the reaction vessel tightly and treat for (30 ± 1) min at (70 ± 2) °C.

Subsequently, add 3 ml aqueous sodium dithionite solution (8.3) for the reductive cleavage of the azo groups to the reaction vessel, then shake vigorously and immediately keep at (70 ± 2) °C for another (30 ± 1) min. Then cool to room temperature (20 °C to 25 °C) within 2 min with a cooling mixture of ice, water and salt.

10.3 Liquid-liquid extraction

Add 1,5 ml of the NaOH solution (8.4) to the reaction solution and shake vigorously. Transfer the reaction solution to the diatomaceous earth column (7.3) and allow it to be absorbed by the column for 15 min.

Meanwhile, add 10 ml *t*-butyl methyl ether (8.5) to the reaction vessel and shake vigorously. After the 15 min period decant the *t*-butyl methyl ether (8.5) onto the top of the column and collect the eluate in a 250 ml round-bottom flask.

Rinse the reaction vessel with 10 ml *t*-butyl methyl ether (8.5) and transfer the solvent to the column. Subsequently, pour 60 ml *t*-butyl methyl ether (8.5) directly onto the column.

For amine detection and quantification, the *t*-butyl methyl ether extract is concentrated to a volume less than 5 ml (not to dryness) with a vacuum rotary at a temperature less than 50 °C and a pressure of approximately

450 mbar[2]. If it is necessary to change to another solvent, remove the remainder of the solvent very carefully by means of a weak flow of inert gas.

NOTE 1 Removal of the solvent (concentration in the rotary vacuum evaporator, evaporation to dryness) can lead to substantial amine losses if performed under uncontrolled conditions.

Make up the extract or residue to 2,0 ml with an appropriate solvent for detection and determination of the amines using chromatography [acetonitrile (8.7), *t*-butyl methyl ether (8.5) or methanol (8.6)] without delay. If the complete analysis cannot be performed within (24 ± 1) h, keep the extract at (–18 ± 3) °C and warm carefully to room temperature before analysis.

NOTE 2 Owing to the matrix, individual amines such as 2,4-diaminotoluene and 2,4-diaminoanisole are likely to exhibit a very poor stability, especially in methanol. Where delays occur in the work routine, it is possible that amines are no longer detectable by the time of instrumental measurement.

10.4 Check of the analytical system

Amine recovery rates shall conform with the following minimum requirements:

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

— amine no. 8: recovery rate 20 %;

— amines nos 18, 19, 23 and 24: recovery rate 50 %;

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

If an amine recovery does not comply with the appropriate minimum requirement, then check the procedure and perform a new test.

11 Chromatographic analyses

The detection of the aromatic amines can be performed using the chromatographic techniques listed in 7.7 and examples described in Annex A. Other validated methods can be used. The quantification of the aromatic amines is performed by means of LC-DAD, LC-MS or GC-MS. Where gas chromatography is used, appropriate internal standards as described in 8.9.4 shall be employed.

If any amine is detected by one chromatographic method, then confirmation shall be made using one or more alternative methods. The result is positive only if both methods give a positive result.

12 Calibration

Use the standard solution (8.9.2) to prepare at least three calibration solutions in a range of 2 µg/ml to 30 µg/ml.

13 Evaluation

13.1 Calculation of amine in the sample

Calculate the amine concentration based on the peak areas of the individual amine components. Calculate the content of the amine as a mass fraction, *w*, in milligrams of the individual component per kilogram of leather material (mg/kg) according to Formula (1):

$$w = \rho_c \times \frac{A_s \times V}{A_c \times m_E} \quad (1)$$

2) 1 bar = 0,1 MPa = 10^5 Pa; 1 MPa = 1 N/mm^2

where

ρ_c is the concentration of the amine in the calibration solution, in micrograms per millilitre (µg/ml);

A_s is the peak area of the amine in the sample solution, in area units;

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

V is the volume of the specimen according to 10.3 (final sample volume), in millilitres (ml) (here 2 ml);

m_E is the mass of the leather specimen, in grams (g).

13.2 Reliability of the method

For the reliability of the method, see Annex B.

14 Test report

The test report shall refer to this official method and give information on at least the following aspects:

a) a reference to this document, i.e. ISO 17234-1:2024;

b) identification of the sample;

c) sampling procedure;

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

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

f) the date of the test;

g) the analytical results for the amines in milligrams per kilogram (see Clause 13), 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 colourants 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 colourants which release one or more of the listed amines.

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

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

Care should be taken in the interpretation of less than 30 mg/kg of amines as these can be due to false-positive results. For the interpretation of results, see Annex C.