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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*

ISO/CEN PARALLEL PROCESSING

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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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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). 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.

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 third edition cancels and replaces the second edition (ISO 17234-1:2015), which has been technically revised.

The main changes to the previous edition are as follows:

- a new [Clause 3](#) added;
- technical changes to [Clause 7](#) and [Clause 8](#);
- changes to [Clause 9](#) and [Clause 10](#) to improve the method;
- [Annex C](#) expanded to give examples of false-positive results, suggested procedures and suggested comments in the test report;
- a new informative [Annex D](#) 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 colorants in dyed leathers —

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

1 Scope

This document specifies a method for determining the use of certain azo colorants which can release certain aromatic amines.

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 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 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 General

Certain azo colorants 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- <i>o</i> -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 | <i>o</i> -aminoazotoluene 4-amino-2',3-dimethylazobenzene 4- <i>o</i> -tolylazo- <i>o</i> -toluidine |
| 6 ^a | 99-55-8 | 612-210-00-5 | 202-765-8 | 5-nitro- <i>o</i> -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- <i>m</i> -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 <i>o</i> -dianisidine |
| 12 | 119-93-7 | 612-041-00-7 | 204-358-0 | 3,3'-dimethylbenzidine 4,4'-bi- <i>o</i> -toluidine |
| 13 | 838-88-0 | 612-085-00-7 | 212-658-8 | 4,4'-methylenedi- <i>o</i> -toluidine |
| 14 | 120-71-8 | 612-209-00-X | 204-419-1 | 6-methoxy- <i>m</i> -toluidine <i>p</i> -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 | <i>o</i> -toluidine 2-aminotoluene |
| 19 | 95-80-7 | 612-099-00-3 | 202-453-1 | 4-methyl- <i>m</i> -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 | <i>o</i> -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 colorants 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 colorants 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 high-performance liquid chromatography (HPLC) using a diode array detector (DAD) or mass selective detector (HPLC-MS), capillary gas chromatography with a mass selective detector (GC-MS) or by capillary electrophoresis with a diode array detector (CE-DAD), or qualitatively with 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 HPLC-DAD or GC-MS.

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

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 colorants and aromatic amines.

6.3 National and local safety regulations can apply.

7 Apparatus

The usual laboratory equipment and, in particular, the following:

7.1 Suitable reaction vessel, 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 **High-performance liquid chromatography (HPLC)** 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 ± 5) °C.

8.3 **Aqueous sodium dithionite solution**, $\rho = 200$ mg/ml¹⁾, 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

8.9.4 **Internal standard in solution (IS)**, $\rho = 10$ 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;

1) ρ = mass concentration.

— 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.

For the analytical procedure, accurately weigh a representative sample of 1,0 g of this leather sample in the reaction vessel (7.1).

10 Procedure

10.1 Degreasing

Treat 1,0 g of the leather in a closed 50 ml vessel (7.1) with 40 ml *n*-hexane (8.1) in an ultrasonic bath (7.6) at (40 ± 2) °C for 40 min.

Decant the *n*-hexane layer from the leather sample. 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 a quantity of 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. 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, amines can be no longer detectable by the time of instrumental measurement.

10.4 Check of the analytical system

To check the analysis procedure, add a certain quantity of amines to obtain x mg/l as final concentration in a reaction vessel containing 15 ml of buffer solution (8.9).

Then carry out the procedure set out in 10.3.

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.

11 Chromatographic analyses

The detection of the aromatic amines can be performed using the chromatographic techniques listed in 7.7. Other validated methods can be used. The quantification of the aromatic amines is performed by means of HPLC-DAD, HPLC-MS or GC-MS. Where gas chromatography is used, appropriate internal standards as described in ISO 17234-2 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 (mg/kg) of leather material according to Formula (1):

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

where