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

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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.

Annex A
(informative)

Chromatographic analyses

A.1 Preliminary remark

As the chromatographic equipment (7.7) of the laboratories can vary, no generally applicable instructions can be provided for chromatographic analyses. The following parameters have been successfully tested and used.

A.2 Liquid chromatography (LC)

A.2.1 Liquid chromatography/diode array detector (LC-DAD)

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

NOTE 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 document and does not constitute an endorsement by ISO of this product. Equivalent products can be used if they can be shown to lead to the same results.

A.2.2 liquid chromatography/mass selective detector (LC-MS)

Eluent 1:	acetonitrile;
Eluent 2:	ammonium acetate in 1 000 ml of water, 5 mmol, pH 3,0;
Stationary phase:	C18 (3,5 µm); length: 50 mm; inside diameter: 2,1 mm;
Flow rate:	300 µl/min;
Gradient:	see Table A.1;
Column temperature	40 °C;
Injection volume:	2,0 µl;

Detection: quadrupole-trap and/or ion-trap mass detector, scanning mode and/or MS daughter ion MS detection; DAD: for wavelengths, see A.2.1;

Spray gas: nitrogen (bottled/generator);

Ionization: API electrospray positive, fragmentor 120 V.

Table A.1 — Gradient programme

Time min	Eluent 1 %	Eluent 2 %
0	10	90
1,5	20	80
7,5	90	10

A.3 Capillary gas chromatography (GC-MS)

Capillary column: medium polarity, e.g. SE 54 or DB 5, length: 50 m, inside diameter: 0,32 mm, film thickness: 0,5 µm;

Injector system: split/splitless;

Injector temperature: 250 °C;

Carrier gas: helium;

Temperature programme: 70 °C (2 min), 70 °C to 280 °C (at 10 °C/min), 280 °C (5 min);

Injection volume: 1,0 µl, splitless 2 min;

Detection: MS, scan 45 amu to 300 amu.

A.4 Capillary electrophoresis (CE-DAD)

250 µl of the sample solution (10.3) is mixed with 50 µl HCl (c = 0,01 mol/l) and passed through a membrane filter (0,2 µm). This solution is analysed by means of capillary zone electrophoresis.

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

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 spectrograph at 214 nm, 240 nm, 280 nm, 305 nm.

A.5 Thin-layer chromatography (TLC); HPTLC or TLC only for semiquantitative confirmation

A.5.1 General

Plates (HPTLC):	silica gel 60 with fluorescence indicator F254, (20 × 10) cm;
Applied volume:	5 µl, applied as a line with automatic applicator;
Mobile solvent 1:	chloroform/acetic acid (90 + 10) parts per volume.
Plates (TLC):	silica gel 60, (20 × 10) cm, saturated chamber;
Applied volume:	10,0 µl, applied as a dot with an automatic applicator;
Mobile solvent 2:	chloroform/ethyl acetate/acetic acid (60 + 30 + 10) parts per volume;
Mobile solvent 3:	chloroform/methanol (95 + 5) parts per volume;
Mobile solvents 2 and 3:	successively without drying of the plates.
Detection:	1) ultraviolet (UV) lamp;
	2) after successive treatment with reagents 2 and 3, reaction time approximately 5 min;
Reagent 1:	0,1 % $NaNO_2$ in KOH (c = 1 mol/l);
Reagent 2:	0,2 % α-naphthol in KOH (c = 1 mol/l);
Reagent 3:	0,5 % to 1,0 % of ammonium sulphamate in methanol.

A.5.2 Derivatization procedure

After developing the TLC plate, it is dried in air or by a hand-held hot air drier (e.g. hair dryer) for 1 min or 2 min. Next the plate is immersed in reagent 1 for 30 s to 1 min, then immersed in reagent 3 for 30 s to 1 min. The plate is dried like earlier and then immersed in reagent 2 for 1 min. The plate is then dried by a hot air drier. Instead of immersion, spraying the reagents using an atomizer is also possible.

Annex B
(informative)

Reliability of the method

The data indicated in Table B.1 were obtained in an interlaboratory collaborative trial on different kinds of leathers. The data were obtained by using LC with DAD. The leather specimens were ground according to ISO 4044. For liquid-liquid extraction Merck™ columns, type EXtrelut® NT201, were used.

Table B.1 — Interlaboratory trial — Precision data

Leather sample	Detected amines	Mean mg/kg	Repeatability r mg/kg	Reproducibility R mg/kg
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

Annex C
(informative)

Assessment guide — Interpretation of analytical results[3)]

C.1 General

C.1.1 General

This annex gives complementary technical guidance but does not question the results obtained following the procedure described in this document.

As the occurrence of the amines in very small amounts can lead to false-positive results, the Regulation REACH 1907/2006/Annex XVII[2] defines a limit value of 30 mg/kg of sample material. This value only applies to a single test specimen.

If the detected amount of amine is over 30 mg/kg, it shall be assumed that an azo colourant which release amines (see Table 1) was used. Below 30 mg/kg, it is at present not possible to make a reliable statement on the use of certain azo colourants which release amines (see Table 1) without further information, such as the type and/or purity of the used colourants or the other raw material used.

Assign a specimen with reduced mass as a minor component and give the advice of a greater uncertainty due to lower material homogeneity.

Due to the existence of isomers for some targeted amines (see Table C.1), the laboratory should ensure that the chromatographic and spectral characteristics of the detected analytes are equivalent to the standard amine substances.

C.1.2 Determination of 4-aminoazobenzene

Azo colourants that are able to form 4-aminoazobenzene generate, under the condition of this method, aniline and 1,4-phenylenediamine. Due to detection limits and recovery of 1,4-phenylenediamine, only aniline can be detected. If aniline is detected above 5 mg/kg in a combined test specimen of three parts, then the presence of 4-aminoazobenzene-releasing colourants shall be tested according to ISO 17234-2.

C.1.3 False-positive results

Table C.1 shows substances which can generate false-positive results (including interferences by isomers).

3) Adapted from ISO 14362-1:2017, Annex C.

Table C.1 — Listing of possible reasons for false-positive results

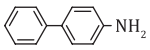
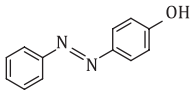
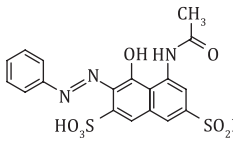
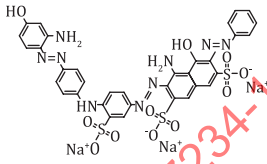
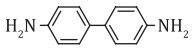
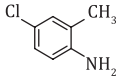
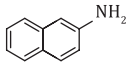
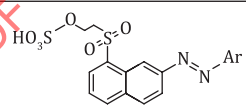
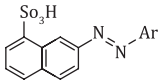
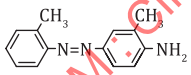
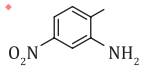
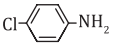
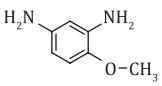
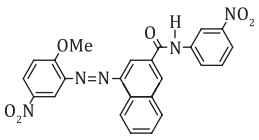
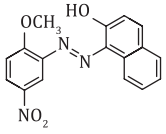
No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
1	92–67–1	biphenyl-4-ylamine 4-aminobiphenyl *p*-xenylamine		Solvent Yellow 7 (SY7) = 4-phenylazophenol = 4-hydroxyazobenzene		Only aminobiphenyl findings are unusual. Such findings can originate from dyestuffs which form 4-aminobiphenyl during the procedure by molecular rearrangement. Three dyestuffs of this kind are listed.
				Acid Red1 (AR1)		
				Direct Black 168		
2	92–87–5	benzidine				No further action needed.
3	95–69–2	4-chloro-*o*-toluidine		10 isomers in all		Take care of the separation of isomers.
4	91–59–8	2-naphthylamine		Reactive Red 174		Desulfonation possible — low commercial relevance.
				Dyes based on Tobias Acid		Take care of impurities of 2-naphthylamine.
				Two isomers in all		Take care of the separation of isomers.
5	97–56–3	*o*-aminoazotoluene 4-amino-2',3-dimethylazobenzene 4-*o*-tolylazo-*o*-toluidine				Detected as *o*-toluidine, look at no. 18.
6	99–55–8	5-nitro-*o*-toluidine 2-amino-4-nitrotoluene				Detected as 2,4-toluylenediamine, look at no. 19.
7	106–47–8	4-chloroaniline		Three isomers in all		Take care of the separation of isomers.
8	615–05–4	4-methoxy-*m*-phenylenediamine 2,4-diaminoanisole		Pigment Red 23		Two reduction steps: 1) step to 2-methoxy-5-nitroaniline; 2) step to 4-methoxy-*m*-phenylene-diamine. Two dyes with 2-methoxy-5-nitroaniline azo bounded are listed beside (see C.2.1.1 and C.2.2.2).
				Pigment Orange 3		
				Six isomers in all		Take care of the separation of isomers.

Table C.1 *(continued)*

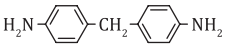
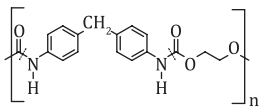
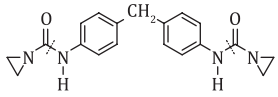
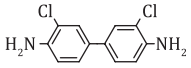
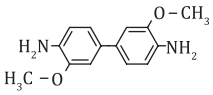
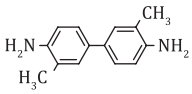
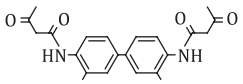
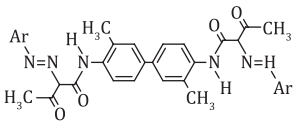
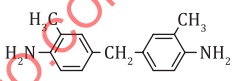
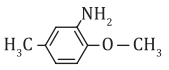
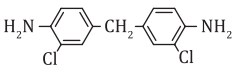
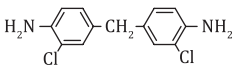
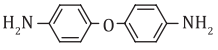
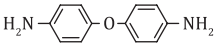
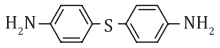
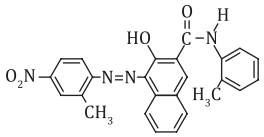
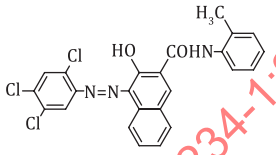
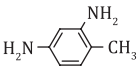
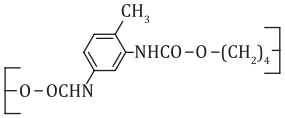
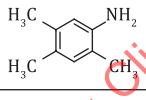
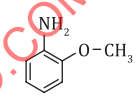
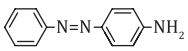
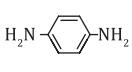
No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
9	101–77–9	4,4'-methylene-dianiline 4,4'-diaminodi-phenyl-methane		Polyurethane polymers of 4,4'-methylenedi-phe-nyl-diisocyanate (MDI)		Foams and print fixing, prepoly-mers, high tem-perature cleavage confirm GC result by LC technique.
				N,N'-(methylene-di-*p*-pheny-lene) bis (aziridine-1-carbox-amide)		Cross-linking auxiliary for print applications.
10	91–94–1	3,3'-dichloro-benzidine 3,3'-dichloro-biphenyl-4,4'-ylene-diamine				No further action needed. For information, combinations of Pigment Black 7 with Pigment Orange 13 or Pig-ment Orange 34 have been known to release the con-cerned amine.
11	119–90–4	3,3'-dimethoxy-benzidine *o*-dianisidine				No further action needed.
12	119–93–7	3,3'-dimethyl-benzidine 4,4'-bi-*o*-tolui-dine		CI Azoic Coupling Component 5		High tempera-ture cleavage of amides, confirm GC result by LC technique.
				Dyes on base of CI Azoic Coupling Compo-nent 5		Dyes on base of CI Azoic Coupling Component 5, high tempera-ture cleavage of amides, confirm GC result by LC technique.
13	838–88–0	4,4'-methyl-ene-di-*o*-tolu-idine				No further action needed (note that a compound with similar MS-spec-tra but different retention time is possible).
14	120–71–8	6-methoxy-*m*-toluidine *p*-cresidine		10 isomers in all		Take care of the separation of isomers.
15	101–14–4	4,4'-methylene-bis-(2-chloro-aniline) 2,2'-di-chloro-4,4'-meth-ylene-dianiline		4,4'-methyl-ene-bis-(2-chloro-an-iline) 2,2'-di-chloro-4,4'-methyl-ene-dianiline		The amine itself is a curing agent for TDI-polyure-thanes, polyure-thane-resins and epoxy–resins.
16	101–80–4	4,4'-oxydianiline		4,4'-oxydianiline		The amine itself is a curing agent for epoxy-resins and thermosetting resins, using vis-cous pre-polymer compositions, which changes ir-reversibly into an infusible polymer network by curing induced by heat or radiation.

Table C.1 *(continued)*

No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
17	139–65–1	4,4'-thiodianiline				No further action needed.
18	95–53–4	*o*-toluidine 2-aminotoluene		Pigment Red 12		High temperature cleavage of amides in GC injector two dyestuffs of this kind are listed besides. Confirm GC result by LC technique.
				Pigment Red 112		
				Three isomers in all		Take care of the separation of isomers. Difficult GC-separation, other polarity or slow temperature rate or separation with LC technique.
19	95–80–7	4-methyl-*m*-phenylenediamine 2,4-toluylenediamine 2,4-diaminotoluene		Polyurethane polymers of 2,4-toluylenediisocyanate (TDI)		Foams and print fixing, pre-polymers.
				Six isomers in all		Take care of the separation of isomers.
20	137–17–7	2,4,5-trimethylaniline		Six isomers in all		Take care of the separation of isomers.
21	90–04–0	*o*-anisidine 2-methoxyaniline				High temperature cleavage in GC injector possible. Confirm GC result by LC technique.
				Three isomers in all		Take care of the separation of isomers.
22	60–09–3	4-aminoazobenzene				4-aminoazobenzene is an azo-dyestuff itself named "Solvent Yellow 1." Proceed to ISO 17234-2.
Other relevant amines						
	62–53–3	aniline				Proceed to ISO 17234-2.
	106–50–3	1,4-phenylenediamine		Three isomers in all		Take care of the separation of isomers.

In this context, it is recommended that the analytical results are reported as indicated in C.1.4 and C.1.5.

C.1.4 In the case of levels per amine component ≤ 30 mg/kg

According to the analysis as carried out, azo colourants which can release one or more of certain listed amines (see Table 1) by cleavage of their azo group/s were not detected in the commodity submitted.

C.1.5 In the case of levels per amine component > 30 mg/kg

a) Indication of the amine component/s at levels > 30 mg/kg.

b) The analytical result suggests that the commodity submitted has been manufactured or treated using azo colourant/s, which can release one or more of certain listed amines (see Table 1) by cleavage of their azo group/s.

c) False-positive results are possible and Table C.1 contains a list of possible reasons. When false-positive results are suspected, guidance on procedures and explanations is provided in Clause C.2.

C.2 Guidance on procedures and explanations if false-positive results or aromatic amines from sources other than azo colourants are possible

C.2.1 False-positive results from chromatographic inaccuracy

C.2.1.1 False-positive results from isomers

The analysis of 24 amines with many possible isomers is a challenging assignment (Table C.1). Several amines have isomers which can produce false-positive results if the separation technique is not optimised. Keep in mind the separation of the targeted amine from its isomers. Amines with more than one aromatic ring system can also have isomers, but this is less common, and separation is normally easier. Laboratories are obliged to ensure the correct result.

C.2.1.2 False-positive results from high temperature in GC-injector

The amines 12, 18 and 21 sometimes give false-positive results in GC due to high temperature cleavage of amide bonding of colourants; amines 9 and 19 can give false-positive results due to high temperature cracking polyurethane pre-polymers. Quantitative confirmation of the results with a non-GC technique is necessary.

C.2.2 Aromatic amines resulting from sources other than azo colourants

C.2.2.1 False-positive results from chemical procedure

The amines 9, 15, 16 and 19 could have originated from other sources such as polyurethane, cross linkers and other substances.

A simple procedure to differentiate between azo bound or not is to re-analyse the sample following the procedure in Annex F. If the result is comparable to the one reached by the reductive cleavage, the amine is originated from a source other than azo colourants.

If necessary, the following explanation may be given as an example:

— '*(Name of the amine)* was detected at the level of *(result in mg/kg)* according to the procedure described in ISO 17234-1:2020. However, when the procedure was carried out without the reducing agent, a similar result was obtained. Therefore, the amine originates from a source other than azo colourants. No forbidden azo colourants which release amines (Table 1) have been used.'

C.2.2.2 False-positive results from cleavage

The amines 1, 4 and 8 can be indirectly generated during the procedure (reductive cleavage with dithionite) from some colourants which originally do not contain these amines. No clear distinction between these colourants and forbidden azo colourants, which release amines (Table 1), can be made.

The absence of forbidden azo colourants in the test specimen shall be proved by evidence (e.g. traceability records from dyer or dye manufacturer), based on the information of the dye structure, to qualify the concerned results as false-positive results.

If necessary, the following explanation may be given as an example:

— 'Other sources of the detected amine *(name of the amine)* can contribute to the reported results whose origins cannot be proved analytically in laboratory.'

— 4-aminobiphenyl, 2-naphthylamine, 4-methoxy-m-phenylenediamine: the absence of forbidden azo colourants which release amines (Table 1) cannot be reliably ascertained without additional information, for example the chemical structure of the colourants used.

NOTE 4-aminobiphenyl, 2-naphthylamine: the test specimen product could have been coloured with colourants whose structures contain the amines but not azo bound.

— 4-methoxy-m-phenylenediamine: the test specimen product could have been coloured with an azo colourant whose structure does not contain preformed 4-methoxy-m-phenylenediamine but 2-amino-4-nitroanisole. In the course of the analytical procedure, the azo colourant leads to release 2-amino-4-nitroanisole, which in turn forms 4-methoxy-m-phenylenediamine.

Annex D
(informative)

Procedure for liquid/liquid extraction without diatomaceous earth

D.1 Preliminary remark

This procedure describes a screening method for the amines listed in Table 1 using liquid/liquid extraction without a diatomaceous earth column (7.3). Any detection of a listed amine in amounts more than 5 mg/kg and less than 100 mg/kg shall be reanalysed with the method described in this document using the liquid/liquid extraction with diatomaceous earth columns This annex lists the reagents and apparatus that shall be used additionally to those listed elsewhere in the document. The procedure, instead, is reported in its entirety, including details on sample preparation that are also described elsewhere in this document, to facilitate usability.

A screening method similar to the one described here may be used if it yields comparable results to the method described in this annex.

See Clause 9 for instructions on how to prepare the test specimen.

D.2 Additional reagents used

D.2.1 Sodium chloride.

D.2.2 Calibration solution of amines for daily use.

Dilute from the stock solution (8.9.1 or 8.9.2) to a concentration of ρ = 6,0 µg of each amine per millilitre of an appropriate solvent. For GC-MS analysis, dilute with the internal standard solution (D.2.3.1).

D.2.3 Calibration solutions of amines for quantification, concentration range from 0,8 µg up to 20 µg of each amine per millilitre of an appropriate solvent.

For GC-MS analysis, dilute with the internal standard solution (D.2.3.1).

NOTE It is the responsibility of each laboratory to choose appropriate concentrations for the calibration.

D.2.3.1 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.

D.2.3.2 Internal standard for later eluting amines: benzidine-d8, CAS no. 92890-63-6.

ρ = 5 µg of benzidine-d8/ml in solution D.2.3.1.

Benzidine-d8 (CAS 92890-63-6) is a suitable indicator for interferences in the later part of the GC chromatogram.

NOTE If the confirmation analysis for benzidine is done with DAD or TLC, the use of benzidine-d8, CAS no. 92890–63–6 is not feasible because the peak cannot be separated from the non-deuterated benzidine.

D.3 Additional apparatus used

D.3.1 Horizontal shaker, capable of a frequency of 5 s^{-1} and path length 2 cm to 5 cm.

D.3.2 Centrifuge, more than 3 000 r/min.

D.4 Procedure

D.4.1 Preparing sample

Prepare the test specimen (Clause 9) in order to obtain a total mass of 1 g.

D.4.2 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.

D.4.3 Reductive cleavage

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

The reaction vessel is tightly closed and treated for (30 ± 1) min at (70 ± 2) °C.

Subsequently, 3 ml aqueous sodium dithionite solution (8.3), for reductive cleavage of the azo groups, is added to the reaction vessel, which is then shaken vigorously and immediately kept again at (70 ± 2) °C for another (30 ± 1) min, whereupon it is cooled to room temperature (20 °C to 25 °C).

D.4.4 Separation and concentration of the amines

Add 7 g sodium chloride (D.2.1), 1,5 ml of sodium hydroxide aqueous solution (8.4), IS (8.9.4) and 5 ml of *t*-butyl methyl ether (8.5) to the reaction solution and shake for (15 ± 1) min with a horizontal shaker (D.3.1). For complete phase separation after shaking, it is recommended that the mixture is centrifuged (D.3.2).

If possible, use the upper phase for determining the amines without a concentration step.

For amine detection and quantification (see D.4.6), the *t*-butyl methyl ether extract can be concentrated to approximately 1 ml (not to dryness) at no more than 50 °C. 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.

The extract or residue is immediately taken up to an appropriate solvent, for example acetonitrile (8.7) or *t*-butyl methyl ether (8.5), and analysed without delay. If the complete analysis cannot be performed within 24 h, the specimen shall be kept below −18 °C.

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.

D.4.5 Amine detection and quantification

Amine detection can be performed using the chromatographic techniques listed (7.7). Other validated methods may be used. If any of the aryl amines listed in Table 1 is identified at concentrations between 5 mg/kg and 100 mg/kg, it is necessary to reanalyse the sample using the method described in Clause 10, then at least a three-point calibration curve is built up to quantify amine content. Quantification is performed by means of LC or GC-MS. If, in GC-MS analysis, the recovery of the indicator substance benzidine-d8 (D.2.3.2) is lower than 30 % of the expected value (due to matrix effects or unknown reasons), it is possible that amines have not have been detected. In this case, LC-analysis shall be performed for the following later eluting amines: 2, 9, 10, 11, 12, 13, 15, 16 and 17 (see Table 1).

D.4.6 Check procedure

To check the procedure, degrease 1 g of a leather without amines. Then, carry out the procedure described in 10.2 and 10.3. When adding TCA (8.9.4), add a certain quantity of amines to obtain *x* mg/l as the final concentration. Amine recovery rates shall conform with the following minimum requirements:

amines nos 1 to 4, 7, 9 to 17 and 20 to 21:	70 %
amine no. 8:	20 %
amines nos 18, 19, 23 and 24:	50 %
amines nos 5, 6 and 22:	see footnotes to Table 1
aniline:	70 %