

INTERNATIONAL  
STANDARD

ISO  
17234-1

IULTCS  
IUC 20-1

Third edition  
2020-08

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**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*



Reference numbers  
ISO 17234-1:2020(E)  
IULTCS/IUC 20-1:2020(E)

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Published in Switzerland

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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](http://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](http://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](http://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](http://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 for determining the use of certain azo colourants 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 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<sup>[2]</sup> and GB 20400-2006<sup>[3]</sup> (see [Table 1](#)).

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

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 <sup>a</sup>	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 <sup>a</sup>	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 <sup>b</sup>	60-09-3	611-008-00-4	200-453-6	4-aminoazobenzene
23 <sup>c</sup>	95-68-1	612-027-00-0	202-440-0	2,4-xylidine 2,4-dimethylbenzene-1-amine
24 <sup>c</sup>	87-62-7	612-161-00-X	201-758-7	2,6-xylidine 2,6-dimethylbenzene-1-amine

<sup>a</sup> 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).

<sup>b</sup> 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.

<sup>c</sup> 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 colourants 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  $(70 \pm 2)$  °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  $(70 \pm 2)^\circ\text{C}$ .

8.3 **Aqueous sodium dithionite solution**,  $\rho = 200 \text{ mg/ml}^1$ , 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  $\mu\text{g/ml}$  in ethyl acetate for TLC.

8.9.2 **Stock solution of the amines** ([8.8](#)), 200  $\mu\text{g/ml}$  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  $\mu\text{g}$  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)**,  $\rho = 10 \mu\text{g}$  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;

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1)  $\rho$  = 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  $(40 \pm 2)$  °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  $(70 \pm 2)$  °C to the sample.

Close the reaction vessel tightly and treat for  $(30 \pm 1)$  min at  $(70 \pm 2)$  °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  $(70 \pm 2)$  °C for another  $(30 \pm 1)$  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  $(24 \pm 1)$  h, keep the extract at  $(-18 \pm 3)$  °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 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 (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

- $\rho_c$  is the concentration of the amine in the calibration solution, in micrograms per millilitre ( $\mu\text{g/ml}$ );
- $A_s$  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 sample, 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 17234-1:2020;
- 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  $\leq 30$  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 > 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](#).

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## 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.

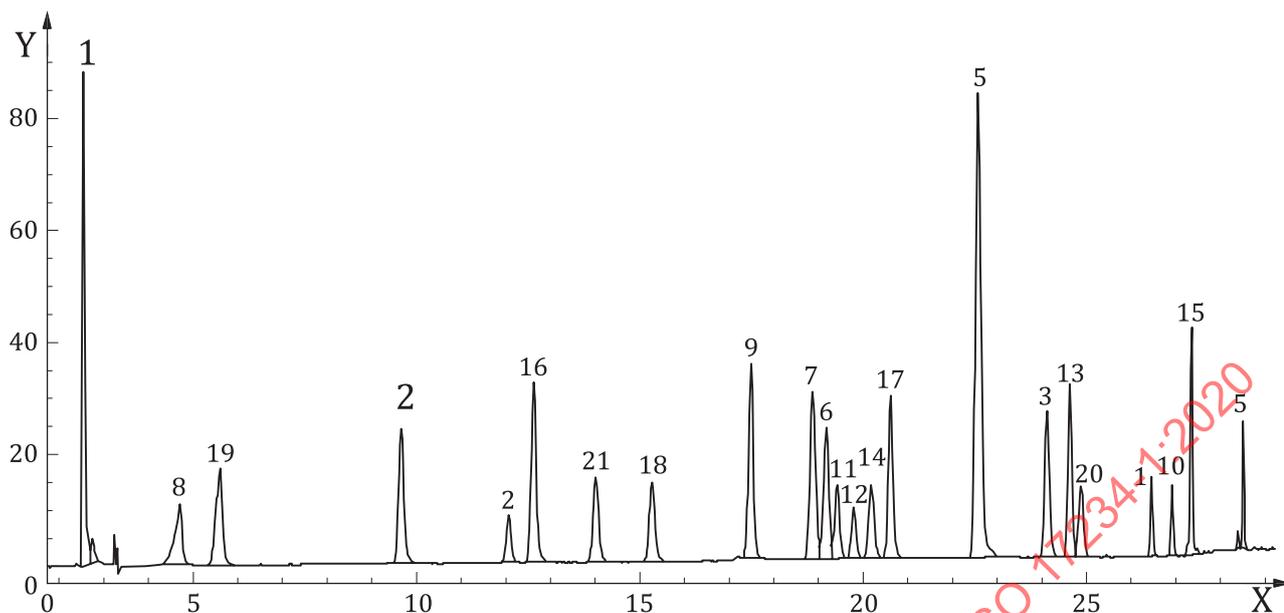
#### A.2 High-performance liquid chromatography (HPLC)

##### A.2.1 High-performance liquid chromatography/diode array detector (HPLC-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 2 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.

See example in [Figure A.1](#).



**Key**

- X time in min
- Y absorbance in mAU at 240 nm
- 1 1,4-phenylenediamine
- 2 aniline

NOTE For aromatic amines 1 to 21, see [Table 1](#) (aromatic amines 23 and 24 are not shown).

**Figure A.1 — HLPC-DAD-chromatogram**

**A.2.2 High-performance liquid chromatography/mass selective detector (HPLC-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- 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 by 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 <sub>2</sub> in KOH ( <i>c</i> = 1 mol/l);
Reagent 2:	0,2 % α-naphthol in KOH ( <i>c</i> = 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 HPLC with DAD. The samples 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	Reproducibility
			<i>r</i> mg/kg	<i>R</i> 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<sup>2)</sup>

#### 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 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 should 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).

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<sup>2)</sup> From ISO 14362-1:2017, Annex C.

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

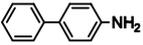
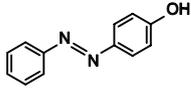
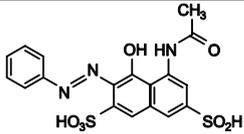
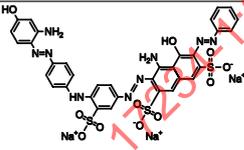
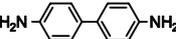
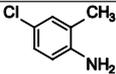
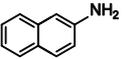
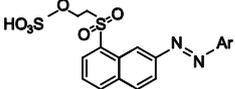
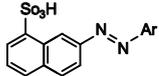
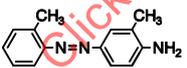
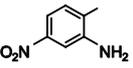
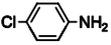
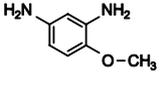
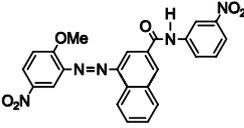
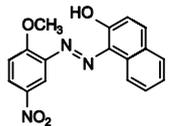
No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
1	92-67-1	biphenyl-4-ylamine 4-aminobiphenyl <i>p</i> -xenyamine		Solvent Yellow 7 (SY7) = 4-phenylazo-phenol = 4-hydroxyazobenzene		Only aminobiphenyl findings are unusual.  Such findings could 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- <i>o</i> -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.  Take care of impurities of 2-naphthylamine.  Take care of the separation of isomers.
				Dyes based on Tobias Acid		
				Two isomers in all		
5	97-56-3	<i>o</i> -aminoazotoluene 4-amino-2',3-dimethylazobenzene 4- <i>o</i> -tolylazo- <i>o</i> -toluidine				Detected as <i>o</i> -toluidine look at no. 18.
6	99-55-8	5-nitro- <i>o</i> -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- <i>m</i> -phenylenediamine 2,4-diaminoanisole		Pigment Red 23		Two reduction steps: 1) step to 2-methoxy-5-nitroaniline, 2) step to 4-methoxy- <i>m</i> -phenylene-diamine.  Two dyes with 2-methoxy-5-nitroaniline azo bounded are listed beside (see C.2.1.1 and C.2.1.2.3).  Take care of the separation of isomers
				Pigment Orange 3		
				Six isomers in all		

Table C.1 (continued)

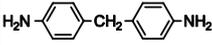
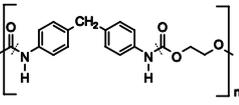
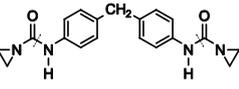
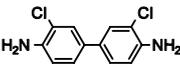
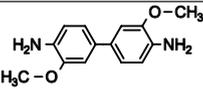
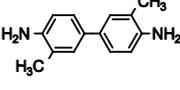
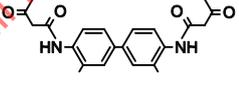
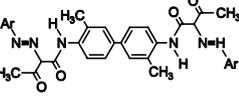
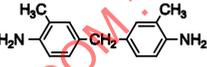
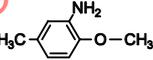
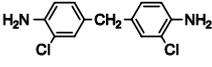
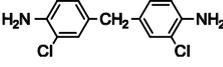
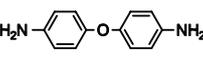
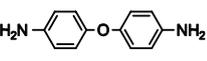
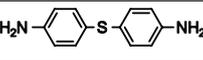
No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
9	101-77-9	4,4'-methylene-dianiline 4,4'-diaminodiphenyl-methane		Polyurethane polymers of 4,4'-methylene-di-phenyl-diisocyanate (MDI)		Foams and print fixing, prepolymers, high temperature cleavage confirm GC result by LC technique.
				N,N'-(methylene-di-p-phenylene) bis (aziridine-1-carboxamide)		Cross-linking auxiliary for print applications
10	91-94-1	3,3'-dichlorobenzidine 3,3'-dichlorobiphenyl-4,4'-ylene-diamine				No further action needed. For information combinations of Pigment Black 7 with Pigment Orange 13 or Pigment Orange 34 have been known to release the concerned amine.
11	119-90-4	3,3'-dimethoxybenzidine o-dianisidine				No further action needed.
12	119-93-7	3,3'-dimethylbenzidine 4,4'-bi-o-toluidine		CI Azoic Coupling Component 5		High temperature cleavage of amides, confirm GC result by LC technique.
				Dyes on base of CI Azoic Coupling Component 5		Dyes on base of CI Azoic Coupling Component 5, high temperature cleavage of amides, confirm GC result by LC technique.
13	838-88-0	4,4'-methylene-di-o-toluidine				No further action needed (note that a compound with similar MS-spectra 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'-dichloro-4,4'-methylene-dianiline		4,4'-methylene-bis-(2-chloro-aniline) 2,2'-dichloro-4,4'-methylene-dianiline		The amine itself is a curing agent for TDI-polyurethanes, polyurethane-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 viscous pre-polymer compositions, which changes irreversibly into an infusible polymer network by curing induced by heat or radiation.
17	139-65-1	4,4'-thiodianiline				No further action needed.

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	
18	95-53-4	<i>o</i> -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- <i>m</i> -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	<i>o</i> -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 follows:

#### C.1.4 In the case of levels per amine component $\leq 30$ 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

- 1) Indication of the amine component/s at levels  $> 30$  mg/kg.

- 2) 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.
- 3) 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 are described in [C.2](#).

## C.2 Guidance on procedure and explanations if false-positive results are possible

### C.2.1 False-positive results from chromatographic problems

#### C.2.1.1 False-positive results from isomers

The analysis of 24 amines and a lot of possible isomers is a challenging assignment ([Table C.1](#)). A lot of amines have isomers which can produce false-positive results if the separation technique is not optimized. Keep in mind the separation of the targeted amine regarding its isomers. Amines with more than one aromatic ring system could 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 sources other than azo colourants

##### C.2.1.2.1 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 and the **amines 9 and 19** due to high temperature cracking polyurethane pre-polymers. Quantitative confirmation of the results with a non-GC technique is necessary.

##### C.2.1.2.2 False-positive results generated from chemical procedure

The **amines 9, 15, 16 and 19** sometimes give false-positive results from other sources such as polyurethane, cross linkers and other substances.

A simple procedure to differentiate between azo bound or not is to do the procedure again with water instead of sodium dithionite solution. If the result is comparable to the one reached by the reductive cleavage, the amine is 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.2.1.2.3 False-positive results from colourants

The **amines 1, 4 and 8** can be indirectly generated during the procedure (reduction cleavage with dithionite) from some colourants which do not contain these amines azo bound. 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 has to 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.'