भारतीय मानक
Indian Standard

IS 582 (Part 5/Sec 1) : 2024
ISO 17234-1 : 2020

चमड़े के रासायनिक परीक्षण की पद्धतियाँ

भाग 5 रंगे चमड़े में विशेष एज़ो रंजकों का निर्धारण

अनुभाग 1 एज़ो रंजकों से उत्पन्न विशेष सुगंधित अमाइन का निर्धारण

(पहला पुनरीक्षण)

Methods of Chemical Testing of Leather

Part 5 Determination of Certain Azo Colorants in Dyed Leathers

Section 1 Determination of Certain Aromatic Amines Derived from Azo Colorants

(First Revision)

ICS 59.140.30

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Leather, Tanning Materials and Allied Products Sectional Committee, CHD 17

NATIONAL FOREWORD

This Indian Standard (First Revision) (Part 5/Sec 1) which is identical to ISO 17234 -1 : 2020 'Leather — Chemical tests for the determination of certain azo colorants in dyed leathers — Part 1: Determination of certain aromatic amines derived from azo colorants' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Leather, Tanning Materials and Allied Products Sectional Committee and approval of the Chemical Division Council.

The Indian Standard IS 582 : 1970 Methods of chemical testing of leather (first revision) prescribes the methods of chemical testing for all types of leathers. The Committee responsible for formulating this standard has decided to harmonize the methods of test prescribed in IS 582 with those prescribed in ISO/IULTCS standards. Accordingly, the committee decided to retain IS 582 and publish the harmonized/ adopted test methods published by ISO/IULTCS in various parts of IS 582 as this standard is widely recognized by the Indian Leather Industry

The committee had further decided to publish the adopted/harmonized standards in the following manner:

a) Wherever an existing test method is being replaced by the corresponding ISO/IULTCS test method, the relevant part will be published with the information in the National Foreword about the method of IS 582 being superseded; and

b) When a new test method is being incorporated in IS 582, the same will be published as a new standard and as a subsequent part/section of IS 582.

This Indian Standard was first published in 2018 as an identical adopting of ISO 17234-1 : 2015 under dual numbering. This section of part 5 specifies a method for determining the use of certain azo colorants which can release certain aromatic amines.

This Indian Standard is published in several parts.The other parts in this series are:

Part 1 Determination of volatile matter

Part 2 Determination of water-soluble matter, water soluble inorganic matter and water–soluble organic matter

Part 3 Determination of sulphated total ash and sulphated water-insoluble ash

Part 4 N-Methyl-2-Pyrrolidone NMP in Leather

Part 5 Determination of certain azo colorants in dyed leathers

Sec 2 Determination of 4-aminoazobenzene

Part 6 Determination of metal content

Sec 1 Extractable etals

Sec 2 Total metal content

Part 7 Quantitative analysis of tanning agents by filter method

Part 8 Determination of the preservative (TCMTB, PCMC, OPP, OIT) content in leather by liquid chromatography

Sec 1 Acetonitrile extraction method

Sec 2 Artificial perspiration extraction method

Part 9 Determination of *p*H and difference figure

Part 10 Determination of chromic oxide content

Sec 1 Quantification by titration

Sec 3 Quantification by atomic absorption spectrometry

Sec 4 Quantification by inductively coupled plasma (ICP)

Part 11 Determination of chromium (VI) content in leather

Sec 1 Colorimetric method

Sec 2 Chromatographic method

Part 12 Determination of nitrogen content and hide substance titrimetric method

Part 13 Determination of total silicon content reduced molybdosilicate spectrometric method

Part 14 Determination of matter soluble in dichloromethane and free fatty acid content

This first revision of the standard has been brought out to adopt the latest version of ISO 17234-1 : 2020. In this revision following modifications have been done:

a) A new Clause **3** added;

b) Changes to Clause **7** 'Apparatus' and Clause **8** ' Reagents';

c) changes to Clause **9** and Clause **10** to improve the method;

d) Annex C Assessment guide — Interpretation of analytical results expanded to give examples of false-Positive results, suggested procedures and suggested comments in the test report; and

e) A new informative Annex D added.

The text of ISO standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are however not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards, which are to be substituted in their places, are listed below along with their degree of equivalence for the editions indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 4044 Leather — Chemical tests — Preparation of chemical test samples	IS 16256 : 2022/ ISO 4044 : 2017 Leather — Chemical tests — Preparation of chemical test samples	Identical
ISO 17234-2 Leather — Chemical tests for the determination of certain azo colorants in dyed leathers — Part 2: Determination of 4-Aminoazobenzene	IS 582 (Part 5/Sec 2) : 2018/ ISO 17234-2 : 2011 Methods of chemical testing of leather: Part 5 Determination of certain azo colorants in dyed leathers, Section 2 Determination of 4-Aminoazobenzene	Identical

The Committee has reviewed the provisions of the following International Standards referred in this adopted standard and has decided that they are acceptable for use in conjunction with this standard.

International Standard	*Title*
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

In this adopted standard, reference appears to certain International Standards where the standard atmospheric conditions to be observed are stipulated which are not applicable to tropical/subtropical countries. The applicable standard atmospheric conditions for Indian conditions are 27 °C ± 2 °C and (65 ± 5) percent, relative humidity and shall be observed while using this standard.

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PART 5 DETERMINATION OF CERTAIN AZO COLORANTS IN DYED LEATHERS

SECTION 1 DETERMINATION OF CERTAIN AROMATIC AMINES DERIVED FROM AZO COLORANTS

(*First Revision*)

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[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 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 ± 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 ± 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

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;

1) ρ = mass concentration.

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

8.10 Water, Grade 3 according to ISO 3696.

9 Sampling and preparation of samples

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

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

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

10 Procedure

10.1 Degreasing

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

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

10.2 Reductive cleavage

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

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

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

10.3 Liquid-liquid extraction

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

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

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

For amine detection and quantification, the *t*-butyl methyl ether extract is concentrated to a volume less than 5 ml (not to dryness) with a vacuum rotary at a temperature less than 50 °C and a pressure of approximately 450 mbar. If it is necessary to change to another solvent, remove the remainder of the solvent very carefully by means of a weak flow of inert gas.

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

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

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

10.4 Check of the analytical system

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

Then carry out the procedure set out in 10.3.

Amine recovery rates shall conform with the following minimum requirements:

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

— amine no. 8: recovery rate 20 %;

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

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

11 Chromatographic analyses

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

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 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 ≤ 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.

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.

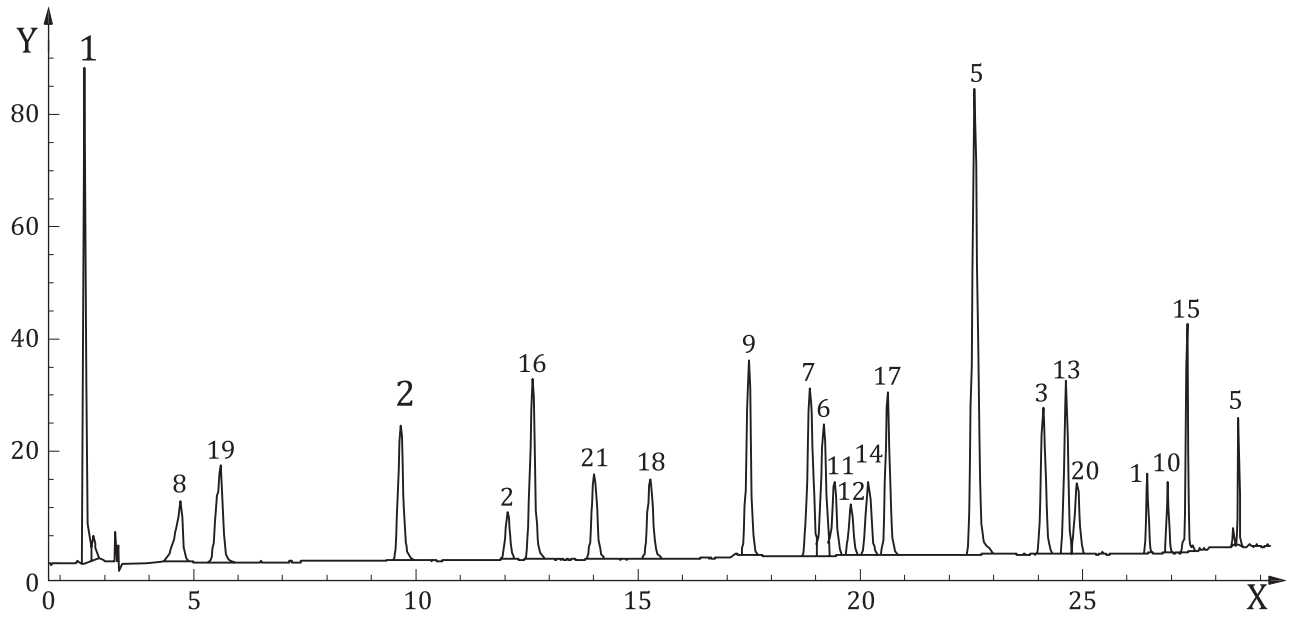
A.2 High-performance liquid chromatography (HPLC)

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 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.

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 % $NaNO_2$ 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 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 *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[2)]

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).

2) From ISO 14362-1:2017, Annex C.

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

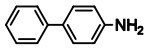
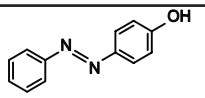
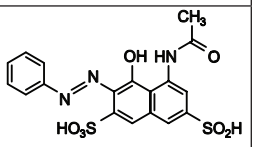
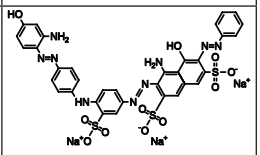
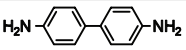
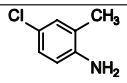
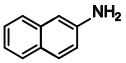
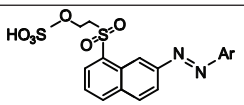
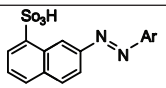
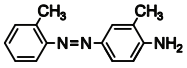
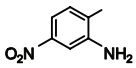
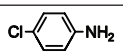
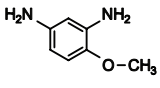
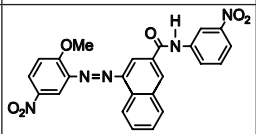
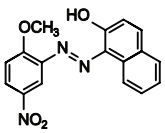
No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
1	92–67–1	biphenyl-4-ylamine 4-aminobiphenyl *p*-xenylamine		Solvent Yellow 7 (SY7) = 4-phenylazo-phenol = 4-hydroxya-zobenzene		Only aminobiphenyl findings are unusual. Such findings could originate from dyestuffs which form 4-aminobiphenyl during the procedure by molecular rear-rangement. 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*-tolyla-zo-*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.2.1.2.3).
				Pigment Orange 3		
				Six isomers in all		Take care of the separation of isomers

Table C.1 *(continued)*

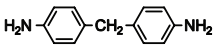
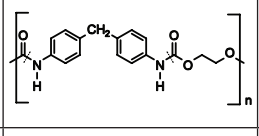
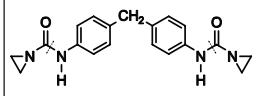
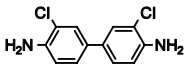
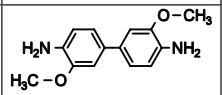
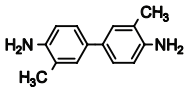
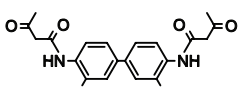
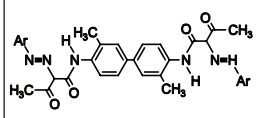
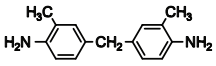
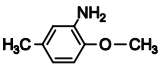
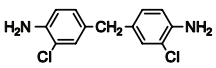
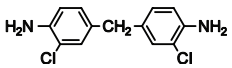
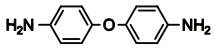
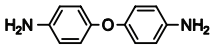
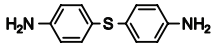
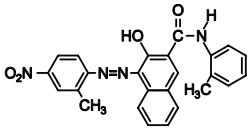
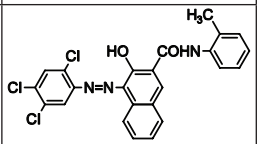
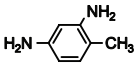
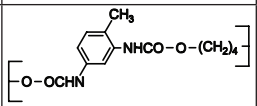
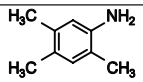
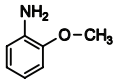
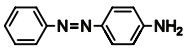
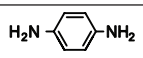
No.	CAS no.	List of aromatic amines		Reasons and substances for false-positive test results		Remarks
		Chemical name	Chemical structure	Chemical name/ number of isomers	Chemical structure	
9	101–77–9	4,4'-methylene-dianiline 4,4'-diaminodi-phenyl-methane		Polyurethane polymers of 4,4'-methylene-di-phenyl-diisocy-anate (MDI)		Foams and print fix-ing, prepolymers, high temperature 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 com-binations 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'-dimethoxy-benzidine *o*-dianisidine				No further action needed.
12	119–93–7	3,3'-dimethyl-benzidine 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 Com-ponent 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'-methyl-ene-di-*o*-toluidine				No further action needed (note that a compound with sim-ilar 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 sepa-ration of isomers.
15	101–14–4	4,4'-methyl-ene-bis-(2-chloro-an-iline) 2,2'-di-chloro-4.4'-methyl-ene-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-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 compo-sitions, which chang-es 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	*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-toluylene-diamine 2,4-diaminotoluene		Polyurethane polymers of 2,4-toluylenediisocy-ante (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-phenylene-diamine		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 ≤ 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.'

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 has to be reanalysed with the method described in this document using the liquid/liquid extraction with diatomaceous earth columns. The description of the procedure is complete including parts for sample preparation that are described in this document to avoid searching for cross-references.

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

See Clause 9 for the application of the test specimen preparation instructions.

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

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

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

D.4.3 Reductive cleavage

Add a quantity of 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 min ± 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 about 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 is to 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, amines can be 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 HPLC 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), amines might not have been detected. In this case, HPLC-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 %

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