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# Textiles — Methods for determination of certain aromatic amines derived from azo colorants —

## Part 1:
## Detection of the use of certain azo colorants accessible with and without extracting the fibres

*Textiles — Méthodes de détermination de certaines amines aromatiques dérivées de colorants azoïques —*

*Partie 1: Détection de l'utilisation de certains colorants azoïques accessibles avec ou sans extraction*

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# Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by the European Committee Standardization (CEN) Technical Committee CEN/TC 248, *Textiles and textile products*, in collaboration with ISO Technical Committee TC 38, *Textiles*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition of ISO 14362-1 cancels and replaces ISO 24362-1:2014, which has been technically revised.

The following is a list of the major technical changes between this edition and ISO 24362-1:2014:

— addition of a new Clause 3 and renumbered;

— changes to Clause 5 "Principle," where the combined method has been removed as it had been found that results were not accurate in certain cases;

— changes to Clause 9 "Test specimen sampling and preparation" to be more explanatory;

— changes to Clause 10 "Procedure" to improve the method, including using xylene as substitute for chlorobenzene (reasons: lower toxicity and lower adverse environmental effect of xylene);

— extension of Annex C "Assessment guide — Interpretation of analytical results" to give examples for false-positive results, suggested procedures and suggested comments in test report;

— addition of Annex G "Pigments."

A list of all parts in the ISO 14362 series can be found on the ISO website.

# Textiles — Methods for determination of certain aromatic amines derived from azo colorants —

## Part 1:
## Detection of the use of certain azo colorants accessible with and without extracting the fibres

## 1 Scope

This document describes a method to detect the use of certain azo colorants that may not be used in the manufacture or treatment of certain commodities made of textile fibres and that are accessible to reducing agent with and without extraction.

Azo colorants accessible to reducing agent without extraction are those used to colour with pigments or to dye

- cellulosic fibres (e.g. cotton, viscose),
- protein fibres (e.g. wool, silk), and
- synthetic fibres (e.g. polyamide, acrylic).

Azo colorants accessible with extraction are those used to dye man-made fibres with disperse dyes. The following man-made fibres can be dyed with disperse dyes: polyester, polyamide, acetate, triacetate, acrylic and chlorofibre.

The method is relevant for all coloured textiles, e.g. dyed, printed and coated textiles.

## 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 3696, *Water for analytical laboratory use — Specification and test methods*

## 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:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

## 4 General

Certain azo colorants may release, by reductive cleavage of azo group(s), one or more of the following aromatic amines.

Table 1 — Targeted aromatic amines[c]

| No. | CAS number | Index number | EC number | Substances |
|---|---|---|---|---|
| 1 | 92-67-1 | 612-072-00-6 | 202-177-1 | biphenyl-4-ylamine<br>4-aminobiphenyl<br>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<br>4-amino-2’,3-dimethylazobenzene<br>4-o-tolylazo-o-toluidine |
| 6[a] | 99-55-8 | 612-210-00-5 | 202-765-8 | 5-nitro-o-toluidine<br>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<br>2,4-diaminoanisole |
| 9 | 101-77-9 | 612-051-00-1 | 202-974-4 | 4,4’-methylenedianiline<br>4,4’-diaminodiphenylmethane |
| 10 | 91-94-1 | 612-068-00-4 | 202-109-0 | 3,3’-dichlorobenzidine<br>3,3’-dichlorobiphenyl-4,4’-ylenediamine |
| 11 | 119-90-4 | 612-036-00-X | 204-355-4 | 3,3’-dimethoxybenzidine<br>o-dianisidine |
| 12 | 119-93-7 | 612-041-00-7 | 204-358-0 | 3,3’-dimethylbenzidine<br>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<br>p-cresidine |
| 15 | 101-14-4 | 612-078-00-9 | 202-918-9 | 4,4’-methylene-bis-(2-chloro-aniline)<br>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<br>2-aminotoluene |
| 19 | 95-80-7 | 612-099-00-3 | 202-453-1 | 4-methyl-m-phenylenediamine<br>2,4-toluylendiamine<br>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<br>2-methoxyaniline |
| 22[b] | 60-09-3 | 611-008-00-4 | 200-453-6 | 4-aminoazobenzene |

[a] The CAS-numbers 97-56-3 (No. 5) and 99-55-8 (No. 6) are further reduced to CAS-numbers 95-53-4 (No. 18) and 95-80-7 (No. 19).

[b] Azo colorants that are able to form 4-aminoazobenzene generate, under the condition of this method, aniline (CAS-number 62-53-3) and 1,4-phenylenediamine (CAS – number 106-50-3). Due to detection limits, only aniline may be detected. If aniline is detected above 5 mg/kg, then the presence of these colorants should be tested by ISO 14362-3.

[c] The targeted aromatic amines are the proscribed aromatic amines under Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency.

## 5 Principle

After selection of a coloured test specimen from the textile article, the test specimen is tested according to the method of colorant extraction for disperse dyes and/or the method of direct reduction for the other classes of colorants (pigments and/or dyes).

The application of one of the two methods is based on the nature of the fibre(s) of the test specimen (composed of pure fibre or of fibre blends) and the colour treatment (dyeing or printing process). When the method of the colorant extraction for disperse dyes is carried out, the colorant is first extracted from the fibre in the headspace (see Figure 1) using xylene under reflux. The extract is concentrated and transferred to the reaction vessel with methanol for subsequent reduction with sodium dithionite in a citrate-buffered aqueous solution (pH = 6) at 70 °C.

If the textile specimen is not completely discoloured after xylene extraction, a new test specimen needs to be prepared again and reprocessed with the method for non-extractable classes of the colorants. When the method for non-extractable classes of the colorants is carried out, the test specimen is treated with sodium dithionite in a citrate-buffered aqueous solution (pH = 6) at 70 °C in a closed vessel.

After the reduction, any amine released in the process is transferred to a *t*-butyl methyl ether phase by means of liquid-liquid extraction using diatomaceous earth columns. The *t*-butyl methyl ether extract is then concentrated, and the residue is taken up in an appropriate solvent for detection and determination of the amines using chromatography (see Annex A).

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

If any amine is detected by one chromatographic method, then confirmation shall be made using one or more alternative methods.

## 6 Safety precautions

**WARNING — The substances [amines] listed in Clause 4 are classified as substances known to be or suspected of being human carcinogens.**

**Any handling and disposal of these substances shall be in strict accordance with the appropriate national health and safety regulations.**

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

## 7 Reagents

Unless otherwise specified, analytical grade chemicals shall be used.

### 7.1 Xylene (mixture of isomers) CAS No 1330-20-7.

### 7.2 Acetonitrile.

### 7.3 Methanol.

### 7.4 *t*-butyl methyl ether.

**7.5 Citrate/sodium hydroxide buffer solution,** pH = 6, $c$ = 0,06 mol/l[1].

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[1] $c$ is citrate concentration.

**7.6 Aqueous sodium dithionite solution,** $\rho$ = 200 mg/ml[2] freshly prepared (which means to be used within 1 h).

**7.7 Diatomaceous earth.**

**7.8 Amine substances** — amines 1 to 4, 7 to 21 (as specified in Table 1), and aniline and 1,4-phenylenediamine — all of highest available defined purity standards.

**7.9 Standard solutions.**

**7.9.1 Stock solution of amines** with a concentration of equal to or greater than 300 µg of each amine per millilitre of an appropriate solvent.

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

**7.9.2 Calibration solution of amines for daily use.**

Dilute from the stock solution (7.9.1) to a concentration of $\rho$ = 15,0 µg of each amine per millilitre of an appropriate solvent.

**7.9.3 Calibration solutions of amines for quantification,** concentration range from 2 µg up to 50 µg of each amine per millilitre of an appropriate solvent. If gas chromatography is used, 10 µl of internal standard solution (7.9.4) per millilitre must be added before injection.

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

**7.9.4 Internal standards in solution (IS)**, $\rho$ = 1,0 mg /ml in the appropriate solvent.

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, CAS No.: 636-30-6;
- IS3: anthracene-d10, CAS No.: 1719-06-8.

**7.9.5 Indicator for later eluting amines: benzidine-d8**, CAS No.: 92890-63-6.

$\rho$ = 0,5 mg/ml of benzidine-d8 in solution (7.9.4)

benzidine-d8 (CAS 92890-63-6) is a suitable indicator for interferences in the later part of the GC chromatogram (10.5).

**7.10 Sodium hydroxide aqueous solution**, a mass fraction of 10 %.

**7.11 Grade 3 water**, complying with ISO 3696**.**

## 8 Apparatus

**8.1 Extraction apparatus**, according to Figure 1, consisting of

- coil condenser NS 29/32,
- a hook, made from an inert material to hold the specimen in place so that the condensed solvent drips onto it,

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[2] $\rho$ is the mass concentration.

- — 100 ml round bottom flask NS 29/32, and
- — heating source.

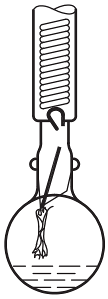


**Figure 1 — Apparatus**

Similar apparatus may be used, if the same results are obtained.

**8.2 Ultrasonic bath.**

**8.3 Reaction vessel** (20 ml to 50 ml) of heat-resistant glass, with tight closure.

**8.4 Heating source** that generates (70 ± 2) °C.

**8.5 Glass or polypropylene column**, inside diameter 25 mm to 30 mm, length 130 mm to 150 mm, packed with 20 g of diatomaceous earth (7.7), 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.

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

Other kinds of evaporation apparatus may be used, e.g. a water bath with a controlled flow of nitrogen over the liquid.

**8.7 Pipettes** in required sizes or variables pipettes.

**8.8 Chromatographic equipment**, selected from the following:

**8.8.1 Thin layer chromatograph (TLC)** or high-performance thin layer chromatograph (HPTLC), including relevant detection.

**8.8.2 High-performance liquid chromatograph (HPLC)** with gradient elution and diode array detector (DAD) or mass selective detector (MS).

**8.8.3 Gas chromatograph (GC)** with flame ionization detector (FID) or mass selective detector (MS).

**8.8.4 Capillary electrophoresis (CE) with diode array detector (DAD).**

**8.8.4.1 Membrane filter PTFE** (polytetrafluoroethylene), pore size 0,2 µm, adapted for capillary electrophoresis (8.8.4)

NOTE A description of the chromatographic conditions is given in Annex A.

# 9 Test specimen sampling and preparation

## 9.1 General

The test specimen shall be selected based on the following criteria:

- parts of the textile article (9.2);
- nature of the fibre components (fibre composition) (9.3 and 9.4);
- coloured materials (9.6), especially when pigments are used (9.5).

Prepare the test specimen by cutting the textile article into strips or pieces (see below) in order to obtain a total mass of 1 g.

For specimens to be submitted to colorant extraction (10.1),

- cut the textile article into strips if the apparatus described in 8.1 is used, or
- cut the textile article into small pieces if another apparatus is used or if the specimens will only be submitted to the reductive cleavage procedure (10.3).

## 9.2 Textile article

If the textile article is a semi-manufactured product, such as yarns, fabrics, etc., cut out test specimens from it.

If the textile article is composed of several parts of textile products, such as a garment, cut out test specimens from all the parts of the textile article, which can be

- principal fabric(s),
- lining(s),
- pocket fabric(s),
- embroideries;
- label(s) for textile article,
- drawstring(s),
- fastener(s),
- false fur, or
- sewing threads.

If the mass of some parts (e.g. labels, sewing threads, embroideries of small size) does not reach the mass (1 g) to be tested, gather identical parts when possible. If the total mass of material is below 0,5 g, this material is defined as a minor component. (See C.1.)

Below 0,2 g of material, the analysis is omitted.

Embroideries shall be weighed with the ground fabric.

## 9.3 Fibre composition

As the application of this document is partly based on the extraction of colorants, identify the nature of the textile components so that the possible use of disperse dyestuffs can be determined.

Table 2 summarizes the four cases:

**Table 2 — Application of colorant extraction for disperse dyes (10.1) in relation to the fibre nature**

| Nature of fibre | Use of disperse dyestuffs | Cases | Colorant extraction for disperse dyes (10.1) necessary? |
|---|---|---|---|
| Natural fibre | No | A | No |
| Man-made fibre | No | B | No |
| | Undetermined | C | Yes |
| | Yes | D | Yes |
| If a fibre is not dyed, the fibre shall not be tested. | | | |

Categories of dyestuffs used in either natural or man-made fibres are explained in Annex D.

## 9.4 Case of the fibre blends

In the case when fibres of different types are mixed, refer to Table 3 in order to decide if application of the colorant extraction for disperse dyes (10.1) shall be applied.

**Table 3 — Application of colorant extraction for disperse dyes (10.1) or other dyes (10.2) in relation to the fibre blends**

| Which procedures shall be applied? | | Other component of the blend | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Component of the blend | A[b] | 10.2 | 10.2 | both 10.1, 10.2 | both 10.1, 10.2 |
| | B[b] | 10.2 | 10.2 | both 10.1, 10.2 | both 10.1, 10.2 |
| | C[b] | both 10.1, 10.2 | both 10.1, 10.2 | both 10.1, 10.2 | both 10.1, 10.2 |
| | D[b] | both 10.1, 10.2 | both 10.1, 10.2 | both 10.1, 10.2 | 10.1[a] |

[a] When pigments are used or when their presence is undetermined, apply 10.2 as well (see 9.5).

[b] See Table 2 for meanings of A, B, C and D.

## 9.5 Coloured materials with pigments

If material is printed or dyed with pigments (Annex G) as well as any material where the usage of pigments is uncertain, the method in 10.2 shall be carried out.

## 9.6 Colours

### 9.6.1 General

All colours shall be tested.

“White” and uncoloured fibres, threads or fabrics are not considered to contain azo colorants and therefore, these parts do not have to be tested. On the other hand, attention should be paid to “pale printed” materials as they can contain azo colorants.

### 9.6.2 Case of colour gathering

Up to three colours may be tested together.

In order to gather three colours, the following rules shall be applied. The rules have been listed in order of preference:

- Select the three colours from the same part of the textile article;
- If the three colours do not come from the same part of the textile article, select these three colours from textile parts made of the same type of textile fibre;

— If the three colours do not come from the same part of the textile article and do not come from the same type of textile fibre, select these three colours from textile parts on which the same procedure shall be applied.

#### 9.6.3 Preparation of the three colour test specimen

Each colour shall have approximately the same weight in order to obtain the total mass of 1 g.

If the result of the combined test specimen is in the range of 5 mg/kg to 30 mg/kg of any of the named amines, separate testing is necessary as the result of testing a single colour test specimen may exceed 30 mg/kg. The quantification limits shall be documented for every amine by internal validation procedures.

## 10 Procedure

### 10.1 Colorant extraction for disperse dyes with xylene

The textile specimen dyed with disperse dyes is kept in the extractor according to 8.1 above 25 ml boiling xylene for approximately 40 min or until solvent drops from the specimen are colourless. The xylene extract is allowed to cool down to room temperature before detaching it from the extractor. If the extract is colourless, which means that the textile specimen is, in fact, not dyed with disperse dyes, it is not necessary to continue the procedure.

Concentrate the xylene extract in the evaporation apparatus at a temperature of 45 °C to 75 °C to dryness. This residue is quantitatively transferred to the reaction vessel with portions of methanol using an ultrasonic bath to disperse the colorant (if the final volume is greater than 2 ml, reduce it to approximately 2 ml), and proceed to the reductive cleavage (10.3). If the textile specimen is not completely decolourised after extraction, additionally, a second test specimen shall be tested according to 10.2.

### 10.2 Textiles coloured with pigments and/or dyed with colorants other than disperse dyes

If the textile specimen contains fibres belonging only to cases A and/or B (9.4) or is coloured with pigments, put the test specimen directly in a reaction vessel.

If the textile specimen is not completely decolourised after the application of the procedure in 10.1, a second test specimen shall be cut and prepared in accordance with 9.1 and put directly in a new reaction vessel.

### 10.3 Reductive cleavage

Add 15 ml of citrate buffer solution (7.5) preheated to 70 °C in the reaction vessel. The reaction vessel is tightly closed and treated for (30 ± 1) min at (70 ± 2) °C.

Subsequently, 3,0 ml aqueous sodium dithionite solution (7.6), for reductive cleavage of the azo groups, are 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) within 3 min.

### 10.4 Separation and concentration of the amines

Add 0,2 ml of the NaOH solution (7.10) to the reaction solution and shake vigorously. Transfer the reaction solution to the diatomaceous earth column (8.5) and allow it to be absorbed by the column for 15 min.

Meanwhile, add 10 ml *t*-butyl methyl ether in the reaction vessel, shake vigorously and after the 15 min period the *t*-butyl methyl ether is decanted (in case of 10.2, with the fibres) onto the top of the column and the eluate is collected in a 100 ml round-bottom flask with standard ground joint or in a glass vessel for an evaporation apparatus (8.6).

The reaction vessel is rinsed with 10 ml *t*-butyl methyl ether and the solvent is transferred to the column. Subsequently, 60 ml *t*-butyl methyl ether is poured directly on the column.

For amine detection and quantification, the *t*-butyl methyl ether extract is concentrated to about 1 ml (not to dryness) at no more than 50 °C. If necessary to exchange 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 are immediately made up to 2,0 ml with acetonitrile or *t*-butyl methyl ether (or methanol only when using capillary electrophoresis) and analysed without delay. If the complete analysis cannot be performed within 24 h, keep the extract 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.

## 10.5 Amine detection and quantification

Amine detection can be performed using the chromatographic techniques listed (8.8). Other validated methods may be used.

If any amine is detected using daily calibration (7.9.2) above 5 mg/kg, the quantification shall be carried out using a calibration curve a multipoint calibration graph (7.9.3) with at least three points.

### 10.5.1 Detection of aniline and 1,4-phenylenediamine (indicators of 4-aminoazobenzene)

Azo colorants that are able to form 4-aminoazobenzene generate, under the condition of this method, aniline and 1,4-phenylenediamine (e.g. C.I. Disperse Yellow 23). Due to detection limits and recovery of 1,4- phenylenediamine, only aniline may be detected. If aniline is detected above 5 mg/kg in test specimens (9.6.2), then the presence of 4-aminoazobenzene releasing colorants shall be tested by ISO 14362-3.

### 10.5.2 In relation to quantification equipment / other sources of false positive results

When GC is used, 10 µl of internal standard solution (7.9.4 and 7.9.5) per ml extract (10.4) shall be added before injection.

If the confirmation analysis for benzidine is done with DAD or TLC, the use of benzidine-d8 (7.9.5) is not feasible because the peak cannot be separated from the non-deuterated benzidine. The extract shall be split in two parts before adding the internal standard solution (7.9.4) to the one for GC-MS analyses.

If, in GC-MS analysis, the recovery of the indicator substance benzidine-d8 (7.9.5) is lower than 30 % of the expected value (due to matrix effects or unknown reasons), amines may not have been detected, then HPLC-analysis shall be performed for the following later eluting amines: 2, 9, 10, 11, 12, 13, 15, 16 and 17 (Table 1). If a test specimen contains benzidine after reductive cleavage, it is suitable to calculate the benzidine content with bendzidine-d8 (7.9.5) as internal standard.

When capillary zone electrophoresis (CE) is used, 200 µl of the extract, with solvent changed to methanol (10.4), is immediately mixed (due to instability of some amines in methanol) 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.

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 all selected methods give a positive result. Before concluding, the case of false results shall be considered.

When a discrepancy between results obtained from different chromatographic techniques is observed, these differences in results could be due to different causes (see Annex C), such as

- false-positive results (e.g. due to high temperature in the inlet, or incomplete separation of isomers as described in Table C.1), and
- false-negative results [e.g. changing solvent (Note 1 in 10.4) or used solvent (Note 2 in 10.4] or interference in matrices (see below).

False-positive results could be due also to amines released from other sources (e.g. polyurethane or cross linker). See Annex C.

If the identified amines have isomers, care should be taken about the correct identification.

## 10.6 Check procedure

### 10.6.1 General

To check the procedure, a volume of 100 µl of the amine stock solution (7.9.1) (or a volume to give 30 µg of each amine in the reaction vessel) is added in a reaction vessel (8.3) containing 15 ml of the preheated citrate/sodium hydroxide buffer solution (7.5). This check procedure shall be carried out with each batch of samples.

Then the procedure set out in 10.4 and 10.5 is carried out. Quantify this check standard based on the daily calibration (7.9.2).

### 10.6.2 Calibration using internal standard (quantification performed by gas chromatography)

$$\rho_s = \rho_c \times \frac{A_s \times A_{ISC}}{A_c \times A_{ISS}} \times \frac{V_s}{V} \tag{1}$$

where

$\rho_s$ is the concentration of the amine in the specimen solution, in µg/ml;

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

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

$A_{ISS}$ is the peak area of the internal standard in the specimen solution, in area units;

$A_{ISC}$ is the peak area of the internal standard in the calibration solution, in area units;

$V$ is the final specimen volume made up to according to 10.4, in ml;

$V_s$ amine solution volume used for check procedure, in ml;

$\rho_c$ concentration of the amine in the calibration solution in µg/ml.

### 10.6.3 Calibration without internal standard

$$\rho_s = \rho_c \times \frac{A_s}{A_c} \times \frac{V_s}{V} \tag{2}$$

where

$\rho_s$ is the concentration of the amine in the specimen solution, in µg/ml;

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

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

$V$ is the final specimen volume made up to according to 10.4, in ml;

$V_s$ amine solution volume used for check procedure, in ml;

$\rho_c$ concentration of the amine in the calibration solution, in µg/ml.

### 10.6.4 Amine recovery

Amine recovery rates shall comply with the following minimum requirements:

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

NOTE Currently, there is insufficient experience to give minimum requirements for the amines not listed above (e.g. 1,4-phenylenediamine).

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

# 11 Evaluation

## 11.1 General

As stated in 10.5, if any amine is detected using daily calibration (7.9.2) above 5 mg/kg, the quantification shall be carried out using a multipoint calibration graph (7.9.3) with at least three points.

If both 10.1 and 10.2 are applied, two different results are obtained, and then report the highest value of each amine.

Plot a calibration graph of the response against the known standard concentration (corrected for the response for the internal standard if used). From the calibration graph, interpolate the concentration of the amine in µg/ml ($\rho_s$).

### 11.2 Calculation of amine in the sample

The amine level is calculated as mass portion $w$ in mg/kg of the specimen according to the following formula:

$$w = \frac{\rho_\mathrm{s} \times V}{m_\mathrm{E}} \tag{3}$$

where

$\rho_\mathrm{S}$ is the interpolated concentration of the amine, in µg/ml;

$V$ is the final volume of the extract made up to according to 10.4, in ml;

$m_\mathrm{E}$ is the mass of the textile specimen, in g.

### 11.3 Reliability of the method

For the reliability of the method, see Annex B.

## 12 Test report

The test report shall refer to this official method and state at least the following particulars:

a) reference to this document, i.e. ISO 14362-1;

b) kind, origin and designation of the specimen (partial specimen, if applicable);

c) date of receipt and date of analysis;

d) performed sampling procedure and preparation procedure according to 10.1 and/or 10.2;

e) detection method and quantification method;

f) detection limit per amine in mg/kg;

g) results reported as arylamine(s) level in mg/kg.

NOTE 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 instrumental equipment of the laboratories may vary (8.8), no generally applicable instructions can be provided for chromatographic analyses. The following parameters have been successfully tested and used.

## A.2 Thin layer chromatography (TLC)

| | |
|---|---|
| Plates (HPTLC): | silica gel 60 with fluorescence indicator F254, 20 cm × 10 cm |
| Applied volume: | (2 to 5) µl, applied as a dot |
| Mobile solvent 1: | chloroform/acetic acid (90 + 10) parts per volume |
| Reagent 1: | For NOx-formation, put in an empty chamber a beaker with about 1 ml of sulfuric acid and add a small spatula of solid sodium nitrite. Close the chamber with the lid and let the reaction take place. Put the dry plate in the chamber. After 5 min, take it out and dry in a stream of cold air. |
| Reagent 2: | Then spray the plate with a solution of 0,2 % α-naphthol prepared in KOH ($c$= 1 mol/l) in methanol. |
| Detection: | 1) TLC plates with fluorescence indicator F254<br>2) UV lamp and /or after successive treatment with reagents 1 and 2; reaction time approximately 5 min |

| | |
|---|---|
| Plates (TLC): | silica gel 60 with fluorescence indicator F254, 20 cm × 10 cm |
| Applied volume: | 10,0 µl, applied as a line |
| Mobile solvent 2: | chloroform/ethyl acetate/acetic acid (60 + 30 + 10) parts per volume |
| Mobile solvent 3: | chloroform/methanol (95 + 5) parts per volume |
| Mobile solvent 4: | n-butyl acetate/toluene (30 + 70) parts per volume |
| Development: | saturated chamber |
| Mobile solvents 2 and 3: | successively without drying out the plates |
| Detection: | 1) TLC plates with fluorescence indicator F254<br>2) UV lamp and/or after successive treatment with reagents 1 and 2; reaction time approximately 5 min |

Plates (TLC): silica gel 60, (20 × 20) cm$^2$

Applied volume: 10,0 µl, applied as a line

Mobile solvent 2: chloroform/ethyl acetate/acetic acid (60 + 30+ 10) parts per volume

Mobile solvent 3: chloroform/methanol (95 + 5) parts per volume

Mobile solvents 2 and 3: successively without drying of the plates

Development: saturated chamber

## A.3 High-performance liquid chromatography (HPLC)

### A.3.1 High-performance liquid chromatography/diode array detector (HPLC/DAD)

Eluent 1: methanol

Eluent 2: dissolve 0,68 g Potassium dihydrogen phosphate in 1 000 ml water, subsequently add 150 ml methanol

Stationary phase: Zorbax Eclipse XDB C18®3) (3,5 µm); (150 × 4,6) mm

Flow rate: 0,6 ml/min to 2,0 ml/min (flow gradient, see below)

Column temperature: 32 °C

Injection volume: 5 µl

Detection: DAD, spectrograph

Quantification: at 240 nm, 280 nm, 305 nm and 380 nm

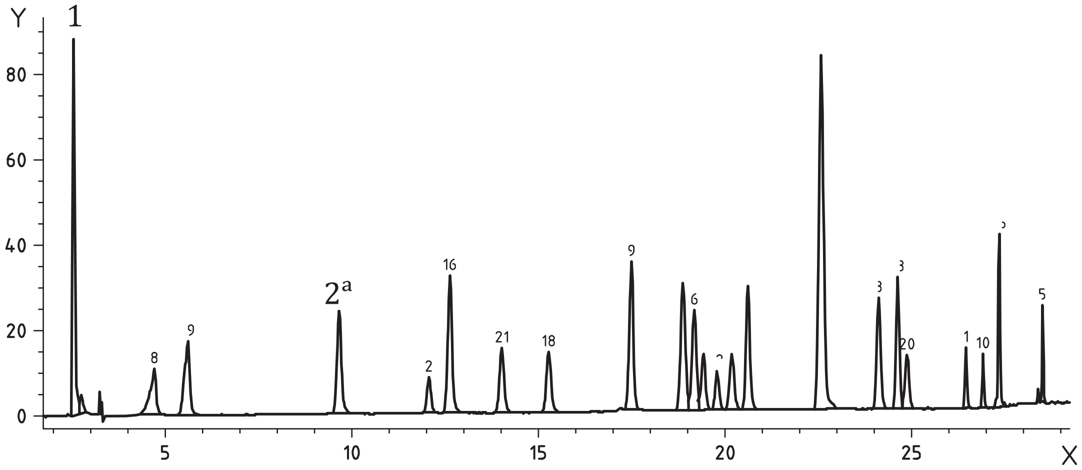
Gradient:

| Time (min.): | Eluent 1 (%): | Flow rate (ml): |
|---|---|---|
| 0,00 | 10,0 | 0,6 |
| 22,50 | 55,0 | 0,6 |
| 27,50 | 100,0 | 0,6 |
| 28,50 | 100,0 | 0,95 |
| 28,51 | 100,0 | 2,0 |
| 29,00 | 100,0 | 2,0 |
| 29,01 | 10,0 | 2,0 |
| 31,0 | 10,0 | 0,6 |
| 35,00 | 10,0 | 0,6 |

See example in Figure A.1.

---

3) Zorbax Eclipse XDB C18® 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.



**Key**

1 1,4-phenylenediamine
2 aniline
X time in min
Y absorbance in mAU at 240 nm
[a] Aromatic amines 1 to 21 (see Table 1).

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

### A.3.2 High-performance liquid chromatography/mass selective detector (HPLC/MS)

| | |
|---|---|
| Eluent 1: | acetonitrile |
| Eluent 2: | 5 mmol ammonium acetate in 1 000 ml water, pH = 3,0 |
| Stationary phase: | Zorbax Eclipse XDB C18® (3,5 µm); (2,1 × 50) mm |
| Flow rate: | 300 µl/min |
| Gradient: | start 10 % eluent 1, increase to 20 % eluent 1 within 1,5 min,<br>linear increase to 90 % eluent 1 within 6 min |
| 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 |
| Spray gas: | nitrogen (bottled/generator) |
| Ionisation: | API electrospray positive, fragmentor 120 V |

### A.4 Capillary gas chromatography/mass selective detector (GC-MS)

| | |
|---|---|
| Capillary column: | DB-35MS (J & W)®, length: 35 m, inside diameter 0,25 mm<br>film thickness: 0,25 µm |
| Injector system: | split or splitless |

Injector temperature: 260 °C

Carrier gas: helium

Temp. programme: 100 °C (2 min), 100 °C to 310 °C (15 °C /min), 310 °C (2 min)

Injection volume: 1,0 µl, split 1:15

Detection: MS

## A.5 Capillary electrophoresis (CE)

Capillary 1: 56 cm, uncoated, inside diameter 50 µm, with extended light path (Agilent®[4])

Capillary 2: 56 cm, coated with polyvinyl alcohol (PVA), inside diameter 50µm, with extended light path (Agilent®)

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 214 nm, 254 nm, spectrograph

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[4] Agilent® 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.

# Annex B
(informative)

# Reliability of the method

The following data have been obtained in a collaborative trial on polyester fabric.[5]

**Table B.1 — Results from an interlaboratory trial A**

| Analytical procedure | Fibre | Amine | $\tilde{x}$ mg/kg | $r$ mg/kg (%) | $s_{(r)}$ mg/kg | $R$ mg/kg (%) | $s_{(R)}$ mg/kg |
|---|---|---|---|---|---|---|---|
| Xylene extraction, HPLC/DAD | polyester | p-chloroaniline | 28,6 | 7,6 (26,6) | 2,7 | 19,8 (69,2) | 7,0 |
| Xylene extraction, GC-MS | polyester | p-chloroaniline | 30,1 | 8,7 (28,9) | 3,1 | 16,8 (55,8) | 5,9 |

where

$r$ is the repeatability;

$R$ is the reproducibility;

$\tilde{x}$ is the mean value;

$s_{(r)}$ is the standard deviation of the repeatability;

$s_{(R)}$ is the standard deviation of the reproducibility.

---

[5] Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG B 82.02-4, Januar 1998: Nachweis der Verwendung bestimmter Azofarbstoffe aus Polyesterfasern.

The following data have been obtained in ring tests on fabrics of wool, cotton and viscose performed by 11 laboratories.[6)]

**Table B.2 — Results from interlaboratory trials**

| Analytical procedure | Fibre | Amine | $\tilde{x}$ mg/kg | $r$ mg/kg (%) | $s_{(r)}$ mg/kg | $R$ mg/kg (%) | $s_{(R)}$ mg/kg |
|---|---|---|---|---|---|---|---|
| HPLC | wool | 3,3’-dimethylbenzidine | 25,9 | 4,9 (18,9) | 1,7 | 12,7 (49,1) | 4,5 |
| HPLC | cotton | benzidine | 29,7 | 5,3 (17,8) | 1,9 | 11,5 (38,7) | 4,1 |
| HPLC | viscose | 3,3’-dimethoxybenzidine | 22,5 | 2,9 (12,9) | 1,0 | 7,9 (35,1) | 2,8 |
| HPLC | wool | 4,4’-diaminodiphenylmethane | 17,7 | 3,0 (16,9) | 1,1 | 7,5 (42,4) | 2,6 |
| HPLC | wool | o-toluidine | 22,6 | 4,4 (19,5) | 1,6 | 13,8 (61,1) | 4,9 |

where

$r$ is the repeatability;

$R$ is the reproducibility;

$\tilde{x}$ is the mean value;

$s_{(r)}$ is the standard deviation of the repeatability;

$s_{(R)}$ is the standard deviation of the reproducibility.

---

[6)] Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG B 82.02-2, Januar 1998/ § 64 LFGB BVL B 82.02-2 , Juni 2004: Nachweis der Verwendung bestimmter Azofarbstoffe aus textilen Bedarfsgegenständen.

# Annex C
(informative)

# Assessment guide — Interpretation of analytical results

## C.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 may 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, but not to combined test specimens (9.6.2).

If the detected amount of amine is over 30 mg/kg, it shall be assumed that an azo colorant (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 colorants (see Table 1) without further information such as the type and/or purity of the used colorants 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.1.1 Determination of 4-aminoazobenzene

Azo colorants that are able to form 4-aminoazobenzene generate, under the condition of this method, aniline and 1,4-phenylenediamine (e.g. C.I. Disperse Yellow 23). Due to detection limits and recovery of 1,4-phenylenediamine, only aniline may 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 colorants should be tested by ISO 14362-3.

### C.1.2 False-positive results

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

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

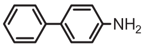
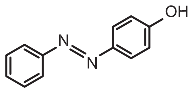
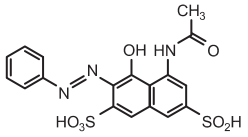
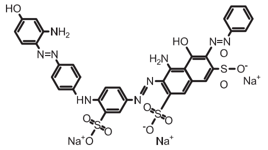
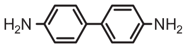
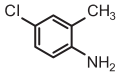
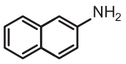
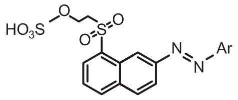
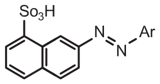
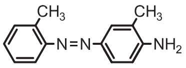
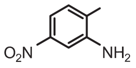
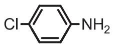
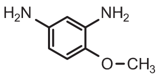
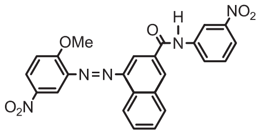
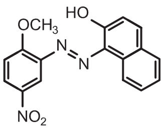
| No. | CAS no. | List of aromatic amines | | Reasons and substances for false-positive test results | | Remarks |
|---|---|---|---|---|---|---|
| | | **Chemical name** | **Chemical structure** | **Chemical name/ Number of isomers** | **Chemical structure** | |
| 1 | 92-67-1 | biphenyl-4-ylamine<br>4-aminobiphenyl<br>p-xenylamine |  | Solvent Yellow 7 (SY7) =4-phenylazophenol = 4-hydroxyazobenzene |  | Only Aminobiphenyl findings are unusual.<br>Such findings could originate from dyestuffs which form 4-aminobiphenyl during the procedure by molecular rearrangement.<br>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<br>4-amino-2′,3-dimethylazobenzene<br>4-o-tolylazo-o-toluidine |  | | | Detected as o-toluidine look at no. 18. |
| 6 | 99-55-8 | 5-nitro-o-toluidine<br>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<br>2,4-diaminoanisole |  | Pigment Red 23 |  | Two reduction steps:<br>1) step to 2-methoxy-5-nitroaniline,<br>2) step to 4-methoxy-m-phenylene-diamine.<br>Two dyes with 2-methoxy-5-nitroaniline azo bounded are listed beside<br>(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)*

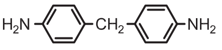
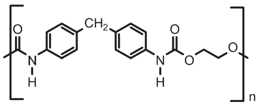
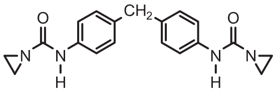
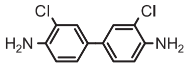
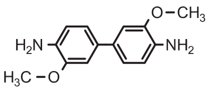
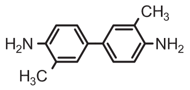
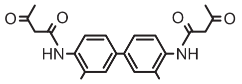
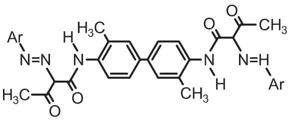
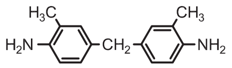
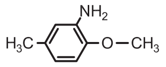
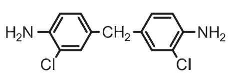
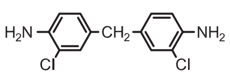
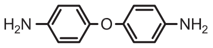
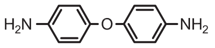
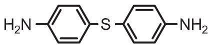
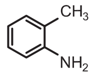
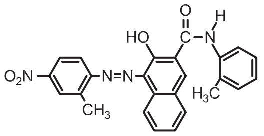
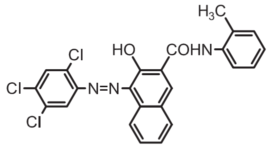
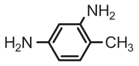
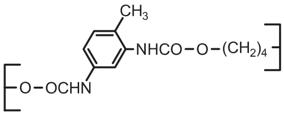
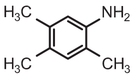
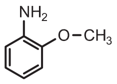
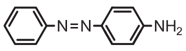
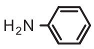
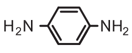
| No. | CAS no. | List of aromatic amines | | Reasons and substances for false-positive test results | | Remarks |
|---|---|---|---|---|---|---|
| | | **Chemical name** | **Chemical structure** | **Chemical name/ Number of isomers** | **Chemical structure** | |
| 9 | 101-77-9 | 4,4’-methylene-dianiline<br>4,4’-diaminodiphenyl-methane |  | polyurethane polymers of 4,4’-methylenedi-phenyl-diisocyanate (MDI) |  | Foams and print fixing, prepolymers, high temperature cleavage confirm GC result by LC technique. |
| | | | | N,N’-(methylene-di-p-pheny-lene) bis (aziridine-1-carboxamide) |  | Cross-linking auxiliary for print applications. |
| 10 | 91-94-1 | 3,3’-dichloro-benzidine<br>3,3’-dichloro-biphenyl-4,4’-ylene-diamine |  | | | No further action needed<br>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’-dimethoxy-benzidine<br>o-dianisidine |  | | | No further action needed. |
| 12 | 119-93-7 | 3,3’-dimethyl-benzidine<br>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<br>(note that a compound with similar MS-spectra but different retention time is possible). |
| 14 | 120-71-8 | 6-methoxy-m-toluidine<br>p-cresidine |  | 10 isomers in all | | Take care of the separation of isomers. |
| 15 | 101-14-4 | 4,4’-methylene-bis-(2-chloro-aniline)<br>2,2’-dichloro-4.4’-methylene-dianiline |  | 4,4’-methylene-bis-(2-chloro-aniline)<br>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 | o-toluidine<br>2-aminotoluene |  | Pigment Red 12 |  | High temperature cleavage of amides in GC injectortwo 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.<br>Difficult GC-separation, other polarity or slow temperature rate or separation with LC technique. |
| 19 | 95-80-7 | 4-methyl-m-phenylenediamine<br>2,4-toluylene-diamine<br>2,4-diaminotoluene |  | polyurethane polymers of 2,4-toluylene-diisocyante (TDI) |  | Foams and print fixing, prepolymers. |
| | | | | 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<br>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.”<br>Proceed to ISO 14362-3. |
| Other relevant amines | | | | | | |
| | 62-53-3 | aniline |  | | | Proceed to ISO 14362-3. |
| | 106-50-3 | 1,4-phenylene-diamine |  | three isomers in all | | Take care of the separation of isomers. |

In this context, it is recommended to report the analytical results as follows:

## C.1.3 In the case of levels per amine component ≤30 mg/kg

According to the analysis as carried out, azo colorants 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.1.4 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 colorant/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 may 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 more seldom and separation is normally easier. Laboratories are obliged to ensure the correct result.

#### C.2.1.2 False-positive results from other sources than azo colorants

#### C.2.1.2.1 False-positive results from high temperature in GC-injector

The **amines 12, 18** and **21** give sometimes false-positive results in GC due to high temperature cleavage of amide bonding of colorants and the **amines 9** and **19** due to high temperature cracking polyurethane prepolymers. 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 differ 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 got by the reductive cleavage, the amine is from another source than azo colorants.

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

"amine "*(name of the amine)*" was detected at the level of *(result in mg/kg)* according to the procedure described in ISO 14362-1:2017. 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 colorants. No forbidden azo colorants which release amines (Table 1) have been used.

#### C.2.1.2.3 False-positive results from colorants

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

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

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

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 colorants which release amines (Table 1) cannot be reliably ascertained without additional information, e.g. the chemical structure of the colorants used.

NOTE 1 4-aminobiphenyl, 2-naphthylamine: the test specimen product can have been coloured with colorants whose structures contain the amines but not azo bound.

4-methoxy-m-phenylenediamine: the test specimen product can have been coloured with an azo colorant whose structure does not contain preformed 4-methoxy-m-phenylenediamine but 2-amino-4-nitroanisole; in the course of the analytical procedure, the azo colorant leads to release 2-amino-4-nitroanisole, which in turn is forming 4-methoxy-m-phenylenediamine.

# Annex D
(informative)

## Explanatory table of dyestuffs used in various textile materials

**Table D.1 — Dyestuffs used in various textile materials**

| Category of colorants | | Dyestuffs | | | | | | | | | | Pigment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Basic | Acid | Chrome | Metal complex | Direct | Disperse | Azoic | Sulfur[a] | Vat[a] | Reactive | |
| Natural fibres | | | | | | | | | | | | |
| Animal fibres | Wool | | xx | xx | xx | (x) | | | | | x | x |
| | Silk | (x) | xx | x | x | (x) | | | (x) | (x) | x | x |
| Cellulose based | Cotton | | | | | xx | | xx | xx | xx | xx | x |
| | Hemp | | | | | | | | | | | |
| | Flax | | | | | | | | | | | |
| | Kapok | | | | | | | | | | | |
| | Sisal | | | | | | | | | | | |
| | Ramie | | | | | | | | | | | |
| | Jute | | | | | | | | | | | |
| Man-made fibres | | | | | | | | | | | | |
| Polyester | | | | | | | xx | | | | | x |
| Polyamide | | | xx | x | xx | (x) | x | | x | x | x | x |
| Triacetate | | | | | | | xx | | | | | x |
| Acetate (2,5) secondary acetate | | | | | | xx | x | (x) | (x) | (x) | xx | x |
| Acrylic | | xx | | | | | (x) | | | | | x |
| Viscose | | | | | | xx | | xx | x | xx | xx | x |
| Chlorofibres | | | | | | | x | | | | | x |

[a] No azo dyes.

x means that the dyestuff category is used

(x) means that the dyestuff category is used in exceptional cases

xx means that the dyestuff category is commonly used

Only disperse dyes are relevant for colorant extraction (10.1). Samples can be screened to determine if disperse dyes are present by extracting fibres in boiling xylene for 20 min. If the solvent is coloured, disperse dyes may have been used; some partly soluble pigments also colourize the solvent.

# Annex E
(informative)

# Procedure for liquid/liquid extraction without diatomaceous earth

## E.1 Preliminary remark

This procedure describes a screening method for amines listed in Table 1 using liquid/liquid extraction without diatomaceous earth column (8.5). Any detection of a listed amine in amounts more than 5 mg/kg and less than 100 mg/kg has to be re-analysed 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 above 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.

## E.2 Additional reagents used

### E.2.1 Calibration solution of amines for daily use

Dilute from the stock solution (7.9.1) to a concentration of $\rho$ = 6,0 µg of each amine per millilitre of an appropriate solvent. For GC-MS analysis, dilute with the internal standard solution (E.2.2.2).

**E.2.2 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 (E.2.2.2).

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

**E.2.2.1 Internal standards in solution (IS)**, ρ = 10 µg of IS/ml of *t*-butyl methyl ether (6.4).

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, CAS No.: 636-30-6;
- IS3: anthracene-d10, CAS No.: 1719-06-8.

**E.2.2.2 Internal standard for later eluting amines**: benzidine-d8, CAS No.: 92890-63-6.

*ρ = 5 µg* of benzidine-d8/ml in solution E.2.2.1.

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.

**E.2.3 Sodium hydroxide aqueous solution** 40 % w/w.

**E.2.4 Sodium chloride.**

## E.3 Additional apparatus used

**E.3.1 Horizontal shaker**, capable of a frequency of 5 s-1, and path length 2 cm to 5 cm.

**E.3.2 Centrifuge**, more than 3 000 r/min.

## E.4 Procedure

### E.4.1 Preparing sample

Prepare the test specimen (9.1) by cutting in order to obtain a total mass of 0,6 g.

### E.4.2 Colorant extraction for disperse dyes with xylene

The textile specimen dyed with disperse dyes is kept in the extractor according to 8.1 above 25 ml boiling xylene for approximately 40 min or until solvent drops are colourless. The xylene extract is allowed to cool down *to room temperature*. If the extract is colourless, it is not necessary to continue the procedure. Concentrate the xylene extract in the evaporation apparatus at a temperature of 45 °C to 75 °C to a small residual quantity. This residue is quantitatively transferred to the reaction vessel with portions of methanol using an ultrasonic bath to disperse the colorant (final volume reduced to approximately 1 ml), and proceed to the reductive cleavage (E.4.4).

If the textile specimen is not completely decolourised after extraction, additionally, a second test specimen shall be tested according to E.4.3.

### E.4.3 Textiles dyed with dyes other than disperse dyes

If the textile specimen contains fibres belonging only to cases A and/or B (9.4) or is not completely decolourised, as described in E.4.2, the second test specimen shall be cut and prepared in accordance with E.4.1 and 9.1 and put it directly in a new reaction vessel.

### E.4.4 Reductive cleavage

Add 8 ml of citrate buffer solution (7.5) preheated to 70 °C in the reaction vessel. The reaction vessel is tightly closed and treated for (30 ± 1) min at (70 ± 2) °C.

Subsequently, 2,0 ml aqueous sodium dithionite solution (7.6), for reductive cleavage of the azo groups, are 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) within 2 min.

### E.4.5 Separation and concentration of the amines

Add 0,5 ml of sodium hydroxide aqueous solution (E.2.3), 7 g sodium chloride (E.2.4), and 3 ml internal standard in solution (E.2.2.1) to the reaction solution and shake for 15 min (±1 min) with a horizontal shaker (E.3.1). For complete phase separation after shaking, it is recommended to centrifuge (E.3.2) the mixture.

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

For amine detection and quantification (see E.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 necessary to exchange to another solvent, remove the remainder of the solvent very carefully by means of a weak flow of inert gas (see 10.4).

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, e.g. acetonitrile or *t*-butyl methyl ether, 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.

### E.4.6 Amine detection and quantification

Amine detection can be performed using the chromatographic techniques listed (8.8). 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 re-analyse 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 (7.9.5) is lower than 30 % of the expected value (due to matrix effects or unknown reasons), amines may not have been detected then HPLC-analysis shall be performed for the following later eluting amines: 2, 9, 10, 11, 12, 13, 15, 16 and 17 (Table 1).

### E.4.7 Check procedure

To check the procedure, 60 µl of the amine stock solution (7.9.1) (or a lower volume to give 18 µg of each amine in the reaction vessel) and 2,0 ml water, instead of aqueous sodium dithionite solution (7.6), are added to a reaction vessel (8.3) containing 8 ml citrate/sodium hydroxide buffer solution (7.5).

Then the procedure set out in E.4.5 and E.4.6 is carried out.

If the specimen solution is not concentrated, the recovery of the different amines is a constant, physical equilibrium; in this case, the check procedure is a part of the method validation of each laboratory.

If it is necessary to concentrate the amines, the check procedure shall be carried out with each batch of samples. Quantify this check standard based on the daily calibration (E.2.1).

Amine recovery rates shall comply with the following minimum requirements:

| | |
|---|---|
| amines No. 1 to 4, 7, 9 to 17 and 20 to 21: | 70 % |
| amine No. 8: | 20 % |
| amines No. 18 and 19: | 50 % |
| amines No. 5,6 and 22: | see footnotes to Table 1 |
| aniline: | 70 % |

# Annex F
(normative)

# Colorants — Methods for determination of certain aromatic amines

## F.1 General

This annex describes a procedure to detect certain aromatic amines directly from colorants.

NOTE Colorants that release greater than 200 mg/kg of an amine can lead to failure of coloured textiles.

## F.2 Principle

The principle is the same as described in Clause 5, except that the extraction stage (10.1 or 10.2) is skipped as the test specimen is a colorant. The reductive cleavage (10.3), the separation and concentration of the amines (10.4), the amine detection and quantification (10.5) and the check procedure (10.6) remain the same, as well as the evaluation (Clause 11). If it is required to analyse the colorant itself, the method in Annex F shall be used.

## F.3 Test specimen preparation

### F.3.1 General

The test specimen is the colorant as it is supplied by the manufacturer.

### F.3.2 Test specimen quantity

Prepare the test specimen from the colorant in order to obtain a mass of 200 mg.

## F.4 Procedure

Put the test specimen in a reaction vessel, add 2,0 ml methanol and apply the procedure as described in Clause 10 but beginning the application of the instructions given from 10.3.

## F.5 Evaluation

The amine level is calculated as mass portion $w$ in mg/kg of the specimen according to Formula (3) where $m_\mathrm{E}$ is the mass of the colorant test specimen, in g.

## F.6 Test report

Report the results as required in Clause 12.

# Annex G
(informative)

# Pigments

## G.1 Pigment dyeing

### G.1.1 Mass dyeing

Mass dyeing is pigment dyeing of synthetic polymers during the extruding process before fibre formation. Typical fibres are polyamide, acrylic, polyesters and polyolefins.

It is impossible to distinguish optical between such a pigment dyed polymer and a disperse dyed one. A good indication is that the pigment dyed polymer is still coloured after extraction with xylene.

### G.1.2 Fibre dyeing

Fibre dyeing is applicable to all fibres (including glass fibres). It is a single-stage process without after treatment which makes it cheap and universal. The pigments are fixed with a glue or binder. With experience, it is possible to distinguish these fibres from disperse dyed fibres with microscopy. It is possible to shade a mass dyed polymer (G.1.1) afterwards with fibre dyeing.

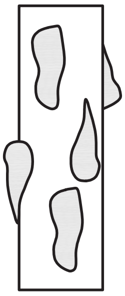


Figure G.1 — Fibre dyeing

### G.1.3 Pigment printing

Pigments are commonly used to produce printed textiles. The application is done with the help of pigment binders, softening agents, flow modifiers, defoamers and thickeners. They are often used to obtain lustrous, metallic and similar effects. Depending on the quality of the binder, a stiff feel of the print is a good indicator that pigments were used as is the low colour fastness to rubbing. The pigments are “glued” to the fibre with resins.

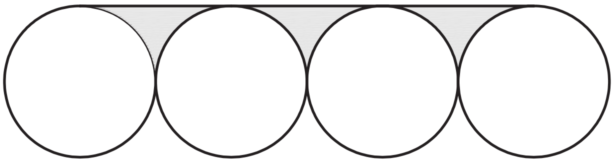


Figure G.2 — Pigment printing

## G.2 Criteria for printed materials

### G.2.1 Criteria for pigment prints

- The print is fixed with binder which remains on the fibre.
- The little particles are bonded to the fibre.
- The handle is stiffer than the unprinted area of the fibre.
- When flexible textiles are stretched, white or bright stripes are visible.
- If you have blends of different fibres, the cheapest procedure for a print is a pigment print.
- Abrasion resistance is worse for pigment prints than for printing with dyes.
- White and brighter colours than the ground material are only possible with pigment prints.

### G.2.2 Criteria for prints with dyes

- The dye is not fixed with binder.
- The dye has penetrated the fibre.
- Flexible textiles normally show, if stretched, no white or bright stripes.
- If you want to print a blend of different fibres, this is difficult because the different fibres show different colour depths.
- Abrasion resistance is much better for prints with dyes than for prints with pigment.

## G.3 Indication for pigments

- For pure polyester, the textile is still coloured after extraction with xylene.
- After extraction with xylene and exchange of solvent to methanol, there is an insoluble coloured residue.
- Criteria for pigment prints (G.2.1).

# Bibliography

[1] ISO 14362-3:2017, *Textiles — Methods for determination of certain aromatic amines derived from azo colorants — Part 3: Detection of the use of certain azo colorants which may release 4-aminoazobenzene*

[2] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency

[3] Schneider, G.: Verwendungsverbot bestimmter Azofarbstoffe für Bedarfsgegenstände: Analytik und Bewertung von Analysenergebnissen Application Ban of Certain Azo colorants for commodities: Analysis and Interpretation of Analytical Results, Deutsche Lebensmittel-Rundschau, 93 (3), 69-102 (1997)

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