



BSI Standards Publication

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

National foreword

This British Standard is the UK implementation of ISO 24362-1:2014.

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A list of organizations represented on this committee can be obtained on request to its secretary.

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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 committee responsible for this document is ISO/TC 38, *Textiles*.

ISO 24362 consists of the following parts, under the general title *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*
- *Part 3: Detection of the use of certain azo colorants, which may release 4-aminoazobenzene*

Introduction

This part of ISO 24362 is based on EN 14362-1:2012 which has been prepared by Technical Committee CEN/TC 248 “Textiles and textile products”, the secretariat of which is held by BSI.

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 part of ISO 24362 describes a procedure 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 a reducing agent with and without extraction.

Azo colorants accessible to a reducing agent without extraction are those used to dye:

- cellulosic fibres (e.g. cotton, viscose);
- protein fibres (e.g. wool, silk);
- 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, modacrylic, aramid and chlorofibre.

For certain commodities made of cellulose and/or protein fibres blended with man-made fibres it is necessary to extract the dye first.

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

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

3 General

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

Table 1 — Aromatic amines subjected

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

^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. The presence of these colorants should be tested by ISO 24362-3..

4 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 dyes.

The application of the combined methods or 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 relevant, if the test specimen is not discoloured during the application of one of the two methods, the other one is carried out.

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 chlorobenzene 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 chlorobenzene extraction, the specimen is added to the reaction vessel with the methanolic solution of the dispersed dye for combined reduction.

When the method for the other classes of the dyes 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 a solvent appropriate 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.

5 Safety precautions

WARNING — The substances [amines] listed in [Clause 3](#) are classified as substances known to be or suspected of being human carcinogens.

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

5.2 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.

5.3 Good laboratory practice should be followed. Wear safety glasses in all laboratory areas and a single-use dust respirator while handling powder colorants.

5.4 Users should comply with any national and local safety regulations.

6 Reagents

Unless otherwise specified, analytical grade chemicals shall be used.

6.1 Chlorobenzene.

WARNING — This is a toxic chemical. To handle chlorobenzene, special care is required to prevent skin contact swallowing and aspiration.

6.2 Acetonitrile.

6.3 Methanol.

6.4 *t*-butyl methyl ether.

6.5 *n*-pentane.

6.6 Citrate/sodium hydroxide buffer solution, pH = 6, c = 0,06 mol/l¹⁾.

6.7 Aqueous sodium dithionite solution, $\rho = 200$ mg/ml²⁾ freshly (daily) prepared.

6.8 Diatomaceous earth.

6.9 Amine substances, amines 1 to 21 (as specified in [Table 1](#)), and aniline and 1,4-phenylenediamine, all of the highest available defined purity standard.

6.10 Standard solutions.

6.10.1 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.

6.10.2 Calibration solution of amines for daily use.

Dilute from the stock solution [6.10.1](#) to a concentration of $\rho = 15,0$ μg of each amine per millilitre of an appropriate solvent.

6.10.3 Calibration solutions of amines for quantification, concentration range from 2 μg up to 50 μg of each amine per millilitre of an appropriate solvent.

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

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

In case of GC-MS analysis, use one of the following internal standards:

- IS1: benzidine-d8, CAS No.: 92890-63-6;
- IS2: naphthalene-d8, CAS No.: 1146-65-2;
- IS3: 2,4,5-trichloroaniline, CAS No.: 636-30-6;
- IS4: anthracene-d10, CAS No.: 1719-06-8.

NOTE If the confirmation analysis for benzidine is done with DAD or TLC the use of IS1: benzidine-d8, CAS No.: 92890-63-6 is not feasible, because the peak cannot be separated from the none deuterated benzidine.

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

6.12 Grade 3 water, complying with ISO 3696:1987.

1) *c* is citrate concentration.

2) ρ is the mass concentration.

7 Apparatus

7.1 **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;
- 100 ml round bottom flask NS 29/32;
- heating source.



Figure 1 — Apparatus

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

7.2 **Ultrasonic bath**, capable of ultrasonic power 160 Watt RMS, with controllable heating equipment.

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

7.4 **Heating source**, capable of maintaining the temperature at $(70 \pm 2) ^\circ\text{C}$.

7.5 **Glass or polypropylene column**, inside diameter 25 mm to 30 mm, length 130 mm to 150 mm, packed with 20 g of diatomaceous earth ([6.8](#)), fitted with a 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.

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

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

7.7 **Pipettes** in required sizes or variable pipettes.

7.8 **Chromatographic equipment** selected from the following:

7.8.1 **Thin layer chromatography (TLC)** or high performance thin layer chromatography (HPTLC) equipment, including relevant detection.

7.8.2 **High performance liquid chromatography (HPLC) equipment**, with gradient elution and diode array detector (DAD) or mass selective detector (MS).

7.8.3 **Gas chromatography (GC) equipment**, with flame ionization detector (FID) or mass selective detector (MS).

7.8.4 Capillary electrophoresis (CE) equipment, with diode array detector (DAD).

NOTE A description of the chromatographic equipment is given in [Annex A](#).

8 Test specimen sampling and preparation

8.1 General

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

- Parts of the textile article;
- Nature of the fibre components (fibre composition);
- Printed materials;
- Colours.

Prepare the test specimen by cutting in order to obtain a total mass of 1 g. For specimens to be submitted to colorant extraction ([9.1](#)) cut into strips (if apparatus described in [7.1](#) is used) or cut into small pieces if other apparatus is used or for specimens to be submitted only to reductive cleavage ([9.3](#)).

8.2 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 that have direct and prolonged contact to skin or mouth, which can be:

- principal fabric(s);
- lining(s);
- pocket fabric(s);
- embroideries;
- label(s) for textile article;
- drawstring(s);
- fastener(s);
- false fur;
- 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 NOTE 2, [Annex C](#).)

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

NOTE If there are omitted portions of sampling because of less than 0,2 g, record the details in the test report.

Embroideries shall be weighed with the ground fabric.

8.3 Fibre composition

As the application of this part of ISO 24362 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 (9.1) in relation to the fibre nature

Nature of fibre	Use of disperse dyestuffs	Cases	Colorant extraction for disperse dyes (9.1) necessary?
Natural fibre	No	A	No
Man-made fibre	No	B	No
	Undetermined	C	Yes
	Yes	D	Yes

NOTE 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](#).

8.4 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 (9.1) shall be applied.

Table 3 — Application of colorant extraction for disperse dyes (9.1) in relation to the fibre blends

Colorant extraction for disperse dyes (9.1) necessary?	Another component of the blend				
	A	B	C	D	
Component of the blend	A	No	No	Yes	Yes
	B	No	No	Yes	Yes
	C	Yes	Yes	Yes	Yes
	D	Yes	Yes	Yes	Yes

NOTE See [Table 2](#) for meanings of A, B, C and D.

8.5 Printed materials

If material is printed with pigments ([Annex D](#)) or dyes other than disperse dyes the method in [9.2](#) has to be used.

8.6 Colours

8.6.1 General

All colours shall be tested.

NOTE “White” is not considered as “colour” and therefore “white” parts do not have to be tested.

8.6.2 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 (9.1 or 9.2) shall be applied.

8.6.3 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 between 5 mg/kg and 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.

9 Procedure

9.1 Colorant extraction for disperse dyes

9.1.1 Extraction of disperse dyes with chlorobenzene

The textile specimen dyed with disperse dyes is kept in the extractor according to (7.1) for 30 min above 25 ml boiling chlorobenzene. The chlorobenzene extract is allowed to cool down *to room temperature* before detaching it from the extractor.

Concentrate the chlorobenzene extract in the evaporation apparatus at a temperature of 45 °C to 60 °C to a small residual quantity. This residue is quantitatively transferred to the reaction vessel with two portions of 1 ml methanol using an ultrasonic bath to disperse the colorant.

9.1.2 Textiles only dyed with disperse dyes

Remove the textile specimen from the extractor and discard it if it is completely made of fibres dyed with disperse dyes and/or becomes decolourised after extraction.

9.1.3 Textiles dyed with disperse dyes and/or other dyes

Remove, from the extractor, the extracted textile specimen if it contains fibres belonging to cases A and/or B (8.4). Remove the solvent by washing the specimen with appropriate solvent e.g. n-pentane (6.5) or t-butyl methyl ether (6.4) and let it dry. If necessary, cut it into small pieces for reductive cleavage. Add the extracted textile specimen to the reaction vessel with the methanolic solution of the dispersed dye (in total 2 ml) for combined reduction.

9.2 Textiles dyed with dyes other than disperse dyes

If the textile specimen contains fibres belonging only to cases A and/or B (8.4) put the test specimen directly in a reaction vessel and add 2 ml methanol (6.3).

9.3 Reductive cleavage

To the reaction vessel add 15 ml of citrate buffer solution (6.6) preheated to 70 °C. The reaction vessel is tightly closed and treated for (30 ± 1) min at (70 ± 2) °C.

Subsequently, 3,0 ml aqueous sodium dithionite solution (6.7), for reductive cleavage of the azo groups, are added to the reaction vessel, which is then shaken vigorously and immediately kept again at $(70 \pm 2) ^\circ\text{C}$ for another (30 ± 1) min whereupon it is cooled to room temperature ($20 ^\circ\text{C}$ to $25 ^\circ\text{C}$) within 2 min.

9.4 Separation and concentration of the amines

Add, to the reaction solution, 0,2 ml of the NaOH solution (6.11) and shake vigorously. Transfer the reaction solution to the diatomaceous earth column (7.5) and allow 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-butylmethyl ether is decanted 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 (7.6).

The reaction vessel is rinsed with 10 ml t-butylmethyl 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 not more than $50 ^\circ\text{C}$. 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) may lead to substantial amine losses if performed under uncontrolled conditions.

The extract or residue are immediately taken up to 2,0 ml of 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, keep the extract below $-18 ^\circ\text{C}$.

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. Where delays occur in the work routine, amines may be no longer detectable by the time of instrumental measurement.

9.5 Amine detection and quantification

Amine detection can be performed using the chromatographic techniques listed (7.8). Other validated methods may be used. 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.

If any of the amines listed in Table 1 is identified, then at least a three point calibration curve is built up to quantify amine content.

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

9.6 Check procedure

9.6.1 General

To check the procedure, 100 μl of the amine stock solution (6.10.1) (or a volume to give 30 μg of each amine in the reaction vessel) and 2,0 ml methanol are added to a reaction vessel (7.3) containing 15 ml of the preheated citrate/sodium hydroxide buffer solution (6.6). This check procedure shall be carried out with each batch of samples.

Then the procedure set out in 9.3 to 9.5 is carried out. Quantify this check standard based on the daily calibration (6.10.2).

9.6.2 Calibration using internal standard (quantification performed by gas chromatography)

$$\rho_s = \rho_c \times \frac{A_s \times A_{isc}}{A_c \times A_{iss}} \times \frac{V_s}{V}$$

where

- ρ_s concentration of the amine in the sample solution in $\mu\text{g/ml}$;
- A_s peak area of the amine in the specimen solution in area units;
- A_c peak area of the amine in the calibration solution in area units;
- A_{iss} peak area of the internal standard in the specimen solution in area units;
- A_{isc} peak area of the internal standard in the calibration solution in area units;
- V final specimen volume made up to according to [9.4](#) in ml;
- V_s amine solution volume used for check procedure, in ml;
- ρ_c concentration of the amine in the calibration solution in $\mu\text{g/ml}$.

9.6.3 Calibration without internal standard

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

where

- ρ_s concentration of the amine in the sample solution in $\mu\text{g/ml}$;
- A_s peak area of the amine in the specimen solution in area units;
- A_c peak area of the amine in the calibration solution in area units;
- V final specimen volume made up to according to [9.4](#) in ml;
- V_s amine solution volume used for check procedure, in ml;
- ρ_c concentration of the amine in the calibration solution in $\mu\text{g/ml}$.

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.

10 Evaluation

10.1 General

If any amine is detected and/or quantified using daily calibration (6.10.2) above 5 mg/kg, the quantification shall be done using a multipoint calibration graph (6.10.3).

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 (ρ_s).

10.2 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 equation:

$$w = \frac{\rho_s \times V}{m_E}$$

where

ρ_s interpolated concentration of the amine, in µg/ml;

V final volume of the extract made up to according to 9.4 in ml;

m_E weight of the textile specimen, in g.

10.3 Reliability of the method

For the reliability of the method, see Annex B.

11 Test report

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

- a) a reference to this part of ISO 24362;
- b) kind, origin and designation of the specimen (partial specimen, if applicable);
- c) date of receipt and date of analysis;
- d) sampling procedure;
- 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 may 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 (7.8), 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)

A.2.1

Plates (HPTLC):	silica gel 60 with fluorescence indicator F254, (20 × 10) cm ² ;
Applied volume	(2 - 5) µl, applied as a dot;
Mobile solvent 1:	chloroform/acetic acid (90 + 10) parts per volume.
Reagent 1:	For NO _x -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 2. UV lamp and /or after successive treatment with Reagents 1 and 2. Reaction time approximately 5 min.

A.2.2

Plates (TLC):	silica gel 60, (20 × 10) cm ² with fluorescence indicator F254;
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 2. UV lamp and/or after successive treatment with reagents 1 and 2, reaction time approximately 5 minutes.

A.2.3

Plates (TLC): silica gel 60, (20 × 20) cm²;
 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 1000 ml water, subsequently add 150 ml methanol
 Stationary phase: Zorbax Eclipse XDB C18 ® (3,5 µm); (150 × 4,6) mm
 Flow rate: 0,6 - 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 [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

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 X 50) mm;
Flow rate:	300 µl/min;
Gradient:	start 10 % eluent 1, increase to 20 % eluent 1 within 1,5 min, 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);
Ionization:	API electrospray positive, fragmentor 120 V.

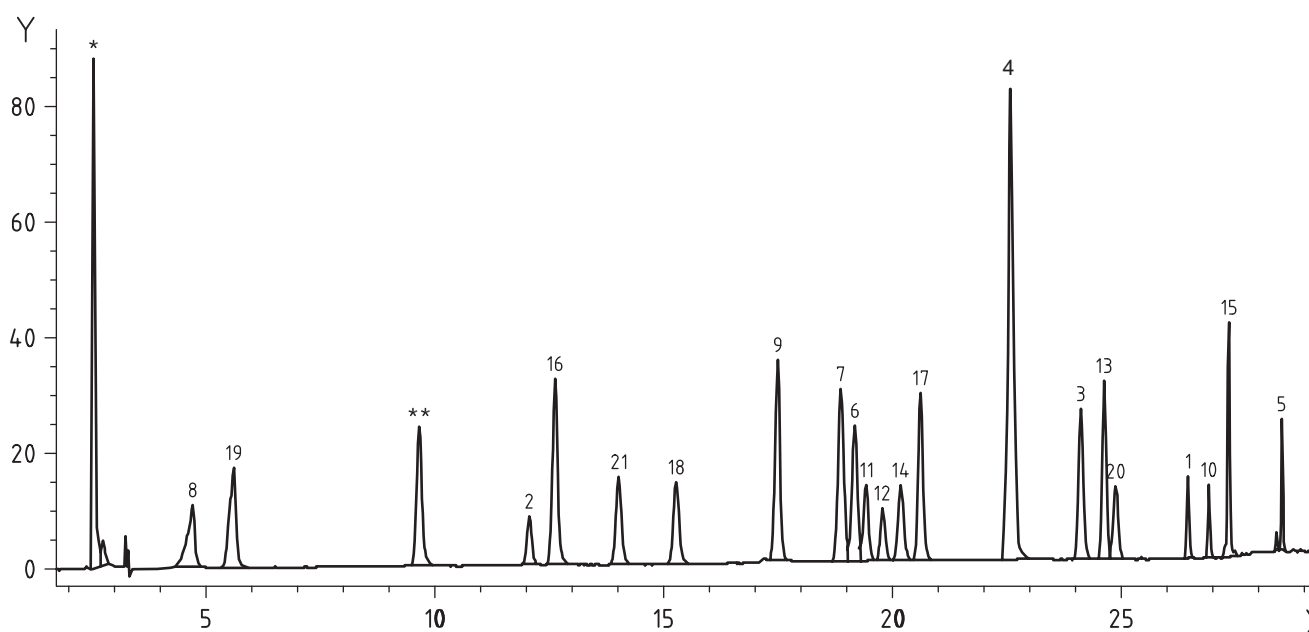
A.4 Capillary gas chromatography/mass selective detector (GC-MS)

Capillary column:	DB-35MS (J and W)®, length: 35 m, inside diameter 0,25 mm, 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)

200 µl of the sample solution (9.4) is mixed with 50 µl HCl ($c = 0,01$ mol/l) and passed through a membrane filter (0,2 µl). This solution is analysed by means of capillary zone electrophoresis.

Capillary 1:	56 cm, uncoated, inside diameter 50 µm, with extended light path (agilent®);
Capillary 2:	56 cm, coated with polyvinyl alcohol (PVA), inside diameter 50 µm, 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.



Key

X time in min

Y absorbance in mAU at 240 nm

* 1,4-phenylenediamine

** aniline aromatic amines 1 - 21 see [Table 1](#)

Figure A.1 — HLPC/DAD-chromatogram

Annex B (informative)

Reliability of the method

The data in [Table B.1](#) have been obtained in a collaborative trial on polyester fabric.

Table B.1 — Results from an interlaboratory trial A

Analytical procedure	Fibre	Amine	\bar{x} mg/kg	r mg/kg (%)	$s(r)$ mg/kg	R mg/kg (%)	$s(R)$ mg/kg
Cl-benzene extraction, HPLC/DAD	polyester	p-chloroaniline	31,6	6,5 (20,6)	2,2	12,7 (40,2)	4,5
Cl-benzene extraction, GC-MS	polyester	p-chloroaniline	31,8	6,8 (21,4)	2,4	10,9 (34,3)	3,8

where
 (r) = repeatability
 (R) = reproducibility
 \bar{x} = mean value
 $s(r)$ = standard deviation of the repeatability
 $s(R)$ = standard deviation of the reproducibility

The data in [Table B.2](#) have been obtained in ring tests on fabrics of wool, cotton and viscose performed by 11 laboratories.

Table B.2 — Results from interlaboratory trials

Analytical procedure	Fibre	Amine	\bar{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) = repeatability
 (R) = reproducibility
 \bar{x} = mean value
 $s(r)$ = standard deviation of the repeatability
 $s(R)$ = standard deviation of the reproducibility

Annex C (informative)

Assessment guide — Interpretation of analytical results

C.1 EU situation

Since the derivation 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 sample material, which is homogenous in matrix and colouring, but not to combined sample of heterogeneous composition.

If the detected amount of amine is over 30 mg/kg it shall be assumed that a certain 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.

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

- a) ***In the case of levels per amine component \leq 30 mg/kg***
 - 1) 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.
- b) ***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:
 - i) 4-aminodiphenyl, 2-naphthylamine, 4-methoxy-m-phenylenediamine: the use of certain azo colorants ([Table 1](#)) cannot be reliably ascertained without additional information, e.g. the chemical structure of the colorants used;
 - ii) 4-aminodiphenyl, 2-naphthylamine: the product from which the sample was taken may have been coloured with colorants whose structures contain the amines but not azo bound;
 - iii) 4-methoxy-m-phenylenediamine: the product from which the sample was taken may 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 will release 2-amino-4-nitroanisole, which in turn will form 4-methoxy-m-phenylenediamine.

NOTE 1 Care should be taken that detected aromatic amines originate from azo colorants and not from other materials such as polyurethane.

NOTE 2 Assign specimen with reduced mass as minor component and give the advice of a greater uncertainty due to lower material homogeneity.

c) ***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. The presence of 4-aminoazobenzene releasing colorants should be tested by ISO 24362-3.

C.2 Korean situation

According to Quality Management & Safety Control of Industrial Products Act, 24 aromatic amines in textile products for infant and textile products have a limited value of 30 mg/kg of sample material. The determination method for those 24 aromatic amines is described in KS K 0147: 2008 *Test method for determination of aryl amine level on the dyestuff and dyed products* and KS K 0734: 2012 *Test method for determination of arylamines content in polyester textiles*. [Table 1](#) shows 22 aromatic amines, however, two more aromatic amines which 2,4-Xylidine and 2,6-Xylidine are included in both test methods of KS K 0147:2008 and KS K 0734:2012.

Aniline or 1,4-phenylenediamine may be detected under reductive cleavage conditions in the test method. In this case, the presence of 4-aminoazobenzene releasing colorants should be tested according to KS K 0734: 2012 that is the same as EN 14362-3.

C.3 Japanese situation

Japan is preparing a law for the restriction of the use of dyestuffs and certain other materials which release aromatic amines defined in this standard. However, the law will exclude amines of No.5 and No. 6 in [Table 1](#), because they are generated in transition state and include 2,4-Xylidine and 2,6-Xylidine additionally.

The Japanese industrial standards (JIS) relating to the test methods for detection of aromatic amines in [Table 1](#), not the same as the above law, are also being developed right now according to ISO 24362-1 and ISO 24362-3.

The limit value for the law and the standard will be less than 30 mg/kg.

Annex D (informative)

Explanatory table of dyestuffs used in various textile materials

D.1 General

Table D.1

Category of colorants		Dyestuffs									Pigment	
		Basic	Acid	Chrome	Metal complex	Direct	Disperse	Azoic	Sulfur ^a	Vata		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
Triacetate							xx					x
Acetate(2,5) secondary acetate						xx	x	(x)	(x)	(x)		x
acrylic		xx					(x)					x
Viscose						xx		xx	x	xx	xx	x
Chlorofibres							x					x
<p>a no azo dyes</p> <p>x means that the dyestuff category is used</p> <p>(x) means that the dyestuff category is used in exceptional cases</p> <p>xx means that the dyestuff category is commonly used</p>												

Only disperse dyes are relevant for colorant extraction (9.1.1) Samples can be screened to determine if disperse dyes are present by extracting fibres in boiling chlorobenzene for 20 min. If the solvent is coloured disperse dyes may have been used.

D.2 Criteria for printed materials

D.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;
- flexible textiles show if you stretch them white or brighter stripes;
- if you have blends of different fibres the cheapest procedure for a print is a pigment print;
- abrasion resistance is worse than printing with dyes;
- white and brighter colours than the ground material are only possible with pigment prints.

D.2.2 Criteria for prints with dyes

- The dye is not fixed with binder;
- the dye has penetrated the fibre;
- flexible textiles show normally if stretching no white or brighter stripes;
- if you want to print a blend of different fibres, this is difficult, because the different fibres show different colour depth;
- abrasion resistance is much better than prints with pigments.

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 ([7.5](#)). Any detection of a listed amine in amounts more than 5 mg/kg and less than 100 mg/kg has to be reanalyzed with the method described in this part of ISO 24362 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 part of ISO 24362 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 8](#) 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 [6.10.1](#) to a concentration of $\rho = 6,0 \mu\text{g}$ of each amine per millilitre of an appropriate solvent. For GC-MS analysis dilute with the internal standard solution ([E.2.3](#)).

E.2.2 Calibration solutions of amines for quantification concentration range from $0,8 \mu\text{g}$ up to $20 \mu\text{g}$ of each amine per millilitre of an appropriate solvent.

For GC-MS analysis dilute with the internal standard solution ([E.2.3](#)).

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

E.2.3 Internal standards in solution (IS), $P = 15 \mu\text{g}$ of IS/ml of *t*-butyl methyl ether ([6.4](#)).

In case of GC-MS analysis, use one of the following internal standards:

- IS1: benzidine-d8, CAS No.: 92890-63-6;
- IS2: naphthalene-d8, CAS No.: 1146-65-2;
- IS3: 2,4,5-trichloroaniline, CAS No.: 636-30-6;
- IS4: anthracene-d10, CAS No.: 1719-06-8.

NOTE If the confirmation analysis is done with DAD or TLC the use of IS1: benzidine-d8, CAS No.: 92890-63-6 is not feasible because the peak cannot be separated from the none deuterated benzidine.

E.2.4 Sodium hydroxide aqueous solution 40 % w/w.

E.2.5 Sodium chloride.

E.3 Additional apparatus used

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

E.3.2 Centrifuge, more than 3 000 rpm.

E.4 Procedure

E.4.1 Colorant extraction for disperse dyes

E.4.1.1 Extraction of disperse dyes with chlorobenzene

The textile specimen dyed with disperse dyes is kept in the extractor according to (7.1) for 30 min above 25 ml boiling chlorobenzene. The chlorobenzene extract is allowed to cool down *to room temperature*.

Concentrate the chlorobenzene extract in the evaporation apparatus at a temperature of 45 °C to 60 °C to a small residual quantity. This residue is quantitatively transferred to the reaction vessel with two portions of 0,5 ml methanol using an ultrasonic bath to disperse the colorant.

E.4.1.2 Textiles only dyed with disperse dyes

Remove, from the extractor, the extracted textile specimen and discard it if it is completely made of fibres dyed with disperse dyes and/or becomes decolourised after extraction.

E.4.1.3 Textiles dyed with disperse dyes and/or other dyes

Remove, from the extractor, the extracted textile specimen if the specimen contains fibres belonging to cases A and/or B (8.4). Remove the solvent by washing the specimen with appropriate solvent e.g. *n*-pentane (6.5) or *t*-butyl methyl ether (6.4) and let it dry. If necessary, cut it into small pieces for reductive cleavage. Add the extracted textile specimen to the reaction vessel with the methanolic solution of the dispersed dye (in total 1 ml) for combined reduction.

E.4.2 Textiles dyed with dyes other than disperse dyes

If the textile specimen contains fibres belonging only to cases A and/or B (8.4) put the test specimen directly in a reaction vessel and add 1 ml methanol (6.3).

E.4.3 Reductive cleavage

To the reaction vessel add 8 ml of citrate buffer solution (6.6) preheated to 70 °C. The reaction vessel is tightly closed and treated for (30 ± 1) min at (70 ± 2) °C.

Subsequently, 3,0 ml aqueous sodium dithionite solution (6.7), 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 minutes.

E.4.4 Separation and concentration of the amines

Add, to the reaction solution, 0,5 ml of Sodium hydroxide aqueous solution (E.2.4), 7 g sodium chloride (E.2.5), 5 ml internal standard in solution (E.2.3) and shake 15 min (±1 min) with a horizontal shaker (E.3.1). For complete phase separation after shaking, it is recommended to centrifuge the mixture.

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

For amine detection and quantification, the *t*-butyl methyl ether extract can be concentrated to about 1 ml (not to dryness!) at not 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) may 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. Where delays occur in the work routine, amines may be no longer detectable by the time of instrumental measurement.

E.4.5 Amine detection and quantification

Amine detection can be performed using the chromatographic techniques listed (7.8). Other validated methods may be used. If any of the aryl amines listed in Table 1 is identified, then at least a three point calibration curve is built up to quantify amine content. Quantification is performed by means of HPLC/DAD or GC-MS.

E.4.6 Check procedure

To check the procedure, 100 µl of the amine stock solution (6.10.1) (or a volume to give 30 µg of each amine in the reaction vessel) 1,0 ml methanol and 3,0 ml water are added to a reaction vessel (7.3) containing 8 ml citrate/sodium hydroxide buffer solution (6.6).

Then the procedure set out in E.4.4 and E.4.5 is carried out.

If the sample 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 Scope

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

F.2 Principle

The principle is the same as describe in [Clause 4](#), except that the extraction stage ([9.1](#) or [9.2](#)) is skipped as the test specimen is a colorant. The reductive cleavage ([9.3](#)), the separation and concentration of the amines ([9.4](#)), the amine detection and quantification ([9.5](#)) and the check procedure ([9.6](#)) remain the same, as well as the evaluation ([Clause 10](#)).

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 9](#) but beginning the application of the instructions given from [9.3](#).

F.5 Evaluation

The amine level is calculated as mass portion w in mg/kg of the specimen according to the equation in [10.2](#) where m_E is the mass of the colorant test specimen, in g.

F.6 Test report

Report the results as required in [Clause 11](#).

Bibliography

- [1] ISO 24362-3, *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] EN 14362-1, *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*
- [3] EN 14362-3, *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*
- [4] 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

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