INTERNATIONAL STANDARD

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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

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

Partie 3: Détection de l'utilisation de certains colorants azoïques susceptibles de libérer du 4-aminoazobenzène

Reference number
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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).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 14362-3 cancels and replaces ISO 24362-3:2014, which has been technically revised.

The following is a list of the major technical changes between this edition and ISO 24362-3:2014:

— addition of a new Clause 3 and renumbered;

— changes to 7.1 to clarify the preparation and use of sodium dithionite solution;

— changes to Clause 9 "Procedure" to improve the method, including using xylene as substitute for chlorobenzene (reasons: lower toxicity and lower adverse environmental effect of xylene).

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 3:
Detection of the use of certain azo colorants, which may release 4-aminoazobenzene

1 Scope

Azo colorants that are able to form 4-aminoazobenzene, generate under the conditions of ISO 14362-1, the amines aniline and 1,4-phenylenediamine. The presence of these 4-aminoazobenzene colorants cannot be reliably ascertained without additional information (e.g. the chemical structure of the colorant used) or without a special procedure.

This document is supplementary to ISO 14362-1 and describes a special procedure to detect the use, in commodities, of certain azo colorants, which may release 4-aminoazobenzene, and that are

— accessible to reducing agent without extraction, particularly concerning textiles made of cellulose and protein fibres (e.g. cotton, viscose, wool, silk), and

— accessible by extracting the fibres (e.g. polyester or imitation leather).

For certain fibre blends, in 9.3 and 9.4 (with and without extraction) may need to be applied.

The procedure also detects 4-aminoazobenzene (Solvent Yellow 1), which is already available as free amine in commodities without reducing pre-treatment.

The use of certain azo colorants, which may release, by reductive cleavage of their azo group(s), one or more of the other aromatic amines listed in the *Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) as regards Annex XVII*, except 4-aminoazobenzene, cannot be determined quantitatively with this method.

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*

ISO 14362-1:2017, *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 Terms and definition

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), 4-aminoazobenzene.

Table 1 — 4-aminoazobenzene[a]

No.	CAS number	Index number	EC number	Substance
22	60–09–3	611–008–00–4	200–453–6	4-aminoazobenzene

[a] Proscribed aromatic amine under Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorization 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 the colorant extraction for disperse dyes and/or the method of the direct reduction for the other classes of colorants (pigments and/or dyes) (see ISO 14362-1).

The textile sample or the residue of the sample extraction is treated with sodium dithionite in an alkaline solution at 40 °C in a closed vessel. 4-aminoazobenzene, which is released in the process, is transferred to a t-butyl methyl ether phase by means of liquid-liquid extraction. An aliquot of the *t*-butyl methyl ether phase is used for analysis. The detection and determination of 4-aminoazobenzene can be performed using chromatography (see Annex A).

If 4-aminoazobenzene is detected by one chromatographic method, then confirmation shall be made using one or more alternative methods.

6 Safety precautions

WARNING — 4-aminoazobenzene is classified as a substance known to be or suspected to be human carcinogen.

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 Aqueous sodium dithionite solution, ρ = 200 mg/ml freshly prepared: rest the solution in a closed vessel for (55 ± 1) min, transfer it into an open glass beaker, rest for 5 min (−0 min, + 5 min) and then use within 10 min.

7.2 Sodium hydroxide aqueous solution, ω = 2 %.[1)]

1) ω = a mass fraction of 2%.

7.3 Methanol.

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

7.5 *t*-butyl methyl ether.

7.6 Sodium chloride.

7.7 4-aminoazobenzene, highest available defined purity standard.

7.8 Internal standards for gas chromatography (IS), e.g. 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 Standard solutions.

7.9.1 Internal standard solution, IS in *t*-butyl methyl ether, ρ = 10,0 µg/ml.

7.9.2 4-aminoazobenzene calibration solution for checking the experimental procedure and preparation of calibration solutions

4-aminoazobenzene in methanol, ρ = 500 µg/ml.

7.10 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 the specimen,

— 100 ml round bottom flask NS 29/32, and

— heating source.

Figure 1 — Apparatus

NOTE Similar apparatus can 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 a temperature of (40 ± 2) °C.

8.5 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.

8.6 Centrifuge, more than 3 000 r/min.

8.7 Pipettes in required sizes or variable pipettes.

8.8 Shaker, ensuring an efficient mixing of the phases.

NOTE Horizontal shaker with minimum frequency of 5 s^{-1}, path length 2 cm to 5 cm has been found suitable.

8.9 Chromatographic equipment, selected from the following:

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

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

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

8.9.4 Capillary electrophoresis (CE) with DAD including: PTFE membrane filter (polytetrafluoroethylene), pore size 0,2 µm, adapted for capillary electrophoresis.

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

9 Procedure

9.1 General

Apply this document to the test specimen that gave a positive result (pre-fail) for aniline and 1,4-phenylenediamine or only aniline using ISO 14362-1:2017, procedures 10.1 or 10.2. Choose procedure 9.3 if there is a pre-fail with ISO 14362-1:2017, 10.1 and procedure 9.4 if there is a pre-fail with ISO 14362-1:2017, 10.2.

9.2 Preparation of test specimens

In the case of fabrics with multi-coloured patterns, the various colours shall be taken into account separately as far as possible. For commodities consisting of various textile qualities, specimens of the various qualities (in terms of fibre and/or colour) shall be analysed separately.

Prepare the test specimen by cutting in order to obtain a total mass of 1 g. For specimens to be submitted to colorant extraction (9.3), cut into strips (if apparatus described in 8.1 is used) or cut into small pieces if other apparatus is used or for specimens to be submitted only to reductive cleavage (9.4 and 9.5).

9.3 Colorant extraction for disperse dyes — Preparation with extraction

If there is a pre-fail result with ISO 14362-1:2017, 10.1, another textile specimen of the same composition is kept in the extractor (8.1) for 30 min to 40 min above 25 ml boiling xylene 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. Remove the textile specimen from the extractor, and discard it.

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 7 ml methanol (7.3) in total, using an ultrasonic bath to disperse the colorant.

The reductive cleavage (9.5) is then carried out.

It is recommended to carry out the transfer in multiple steps, e.g. to add 4 ml of methanol and to dissolve the residue from the glass flask using an ultrasonic bath, then to transfer the suspension quantitatively into the reaction vessel using a pipette, subsequently to rinse three times with 1 ml of methanol and to transfer the solution quantitatively.

For direct determination of a 4-aminoazobenzene-releasing dispersion colorant (e.g. Disperse Yellow 23), an aliquot of this methanolic solution may be immediately used for analysis by LC-DAD-MS.

9.4 Textiles dyed with dyes other than disperse dyes — Preparation without extraction

If there is a pre-fail result with ISO 14362-1:2017, 10.2, put the cut test specimen directly in a reaction vessel.

9.5 Reductive cleavage

A quantity of 9 ml sodium hydroxide solution (7.2) is added to the reaction vessel containing test specimen (9.4) or methanolic solution (9.3). The reaction vessel is tightly closed and shaken vigorously.

Subsequently, 1,0 ml aqueous sodium dithionite solution (7.1) is added for reductive cleavage. The reaction vessel is tightly closed and the mixture is shaken vigorously and immediately kept without shaking at (40 ± 2) °C for exactly 30 min, whereupon it is cooled to room temperature 20 °C to 25 °C within 1 min.

9.6 Separation and concentration of 4-aminoazobenzene

5 ml *t*-butyl methyl ether (7.5) or 5 ml internal standard solution (7.9.1), are added to the reaction solution. Subsequently, 7 g of sodium chloride (7.6) are added. The reaction vessel is tightly closed and the mixture is shaken using the shaker (8.8) for 45 min, ensuring an efficient mixing of the phases.

It is not recommended that the delay time between cooling down and shaking is greater than 5 min. For complete phase separation after shaking, it is recommended to centrifuge the mixture.

For subsequent analysis, an aliquot of the *t*-butyl methyl ether phase is transferred into an appropriate vial which is closed immediately. The detection and determination of 4-aminoazobenzene can be performed using the chromatographic techniques listed in 8.9.

For subsequent analysis, it may be necessary to change the solvent or to concentrate the extract from 9.5 and transfer it to another appropriate solvent (e.g. methanol). Removal of the solvent (concentration in the vacuum rotary evaporator, evaporation to dryness) may lead to substantial loss of 4-aminoazobenzene if not performed under controlled conditions.

If necessary, concentrate the *t*-butyl methyl ether extract to about 1 ml (not to dryness) in a rotary evaporator in a slight vacuum at not more than 50 °C. Then remove the remainder of the solvent very carefully without vacuum by means of a weak flow of inert gas.

If possible, avoid changing the solvent, as in the course of the analytical procedure severe losses of analyte may result due to matrix effects.

Owing to the matrix, 4-aminoazobenzene may exhibit a poor stability. Where delays occur in the work routine, severe losses of analyte may result.

If the complete analysis cannot be performed within 24 h, the test solution shall be kept below −18 °C.

9.7 Calibration solution

9.7.1 Calibration solution for sample preparation without extraction

5 ml *t*-butyl methyl ether (7.5) or 5 ml internal standard solution (7.9.1), respectively are added to 100 µl of the 4-aminoazobenzene calibration solution (7.9.2). This mixture is used for calibration, as the recovery of 4-aminoazobenzene via phase partition according to this procedure is 95 % to 100 %.

If the amine is detected above 5 mg/kg, the quantification shall be carried out using a calibration curve, e.g. as produced in ISO 14362-1.

9.7.2 Calibration solution for sample preparation with extraction

100 µl of the 4-aminoazobenzene calibration solution (7.9.2) are added to 6,9 ml methanol (7.3), 9 ml sodium hydroxide solution (7.2), 1 ml water, 7 g sodium chloride (7.6) and 5 ml *t*-butyl methyl ether (7.5) or 5 ml internal standard solution (7.9.1).

The reaction vessel is tightly closed and the mixture is shaken using the shaker (8.8) for 45 min, ensuring an efficient mixing of the phases. For subsequent analysis, an aliquot is taken out of the *t*-butyl methyl ether phase. The vial for analysis should be closed immediately.

NOTE Due to the high methanol content in the matrix, different recovery rates can be observed. In this case, it is necessary to produce a calibration curve with at least three points with a methanol containing matrix.

9.8 Check of the analytical system

9.8.1 Sample preparation without extraction

To check the procedure, 100 µl of the 4-aminoazobenzene calibration solution (7.9.2) are treated according to 9.5.

4-aminoazobenzene recovery rate shall be a minimum of 60 %.

9.8.2 Sample preparation with extraction

To check the procedure, 100 µl of the 4-aminoazobenzene calibration solution (7.9.2) are added to 6,9 ml methanol. This mixture is treated according to 9.5.

4-aminoazobenzene recovery rate shall be a minimum of 60 %.

9.9 Chromatographic analyses

4-aminoazobenzene detection can be performed using the chromatographic techniques listed in 8.9. Other validated methods may be used. If this 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.

10 Evaluation

10.1 Calculation

The amount of 4-aminoazobenzene is usually calculated by means of a software program. The calculation can also be carried out manually as described in Annex B.

10.2 Reliability of the method

For the reliability of the method, see Annex C.

11 Test report

The test report shall state at least the following particulars:

a) reference to this document, i.e. ISO 14362-3;

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 9.3 or 9.4;

e) detection method and quantification method;

f) results reported as level and detection limit of 4-aminoazobenzene in mg/kg.

Care should be taken in the interpretation of concentrations of less than 30 mg/kg of 4-aminoazobenzene (see Annex D).

Annex A
(informative)

Chromatographic analyses

A.1 Preliminary remark

As the instrumental equipment (8.9) of the laboratories may vary, 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
Development:	saturated chamber
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
Reagent 1:	For NOx-formation, put in an empty chamber a beaker with about 1 ml of sulphuric 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 1 M in methanol
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
	2) UV lamp and/or after successive treatment with reagents 1 and 2 (A.2), reaction time approximately 5 min

Plates (TLC):	silica gel 60, 20 cm × 20 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 solvents 2 and 3:	successively without drying of the plates
Development:	saturated chamber
Detection:	successive treatment with reagents 1 and 2 (A.2), reaction time approximately 5 min

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®[a] (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, 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

[a] 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.

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®[a] (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, 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] 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.

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

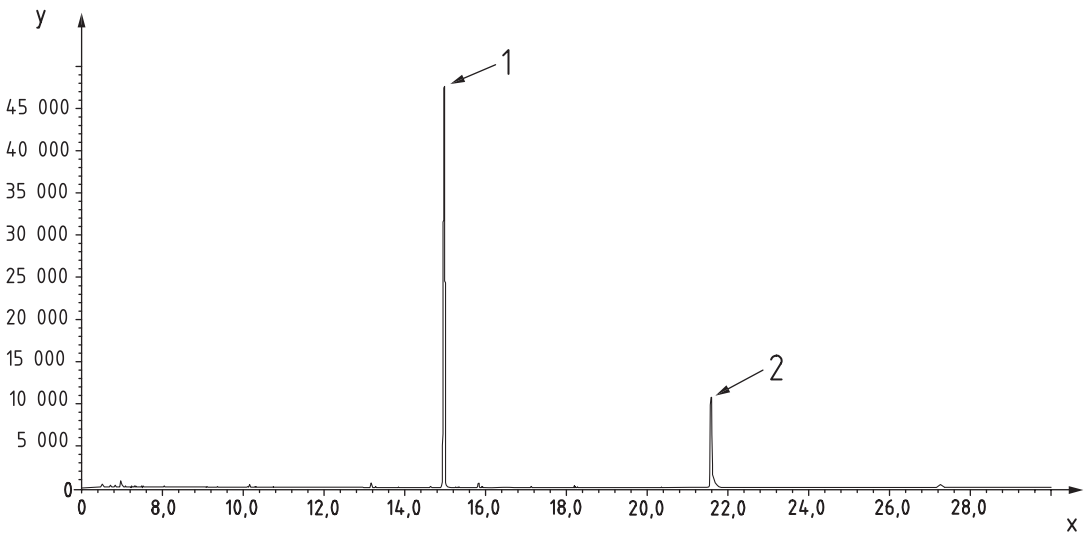
Capillary column:	DB-35MS (J and W)®,[a] length: 35 m, inside diameter 0,25 mm, film thickness: 0,25 µm
Injector system:	split or splitless
Injector temperature:	260 °C
Carrier gas:	Helium
Temp. programme:	100 °C (2 min), 100 °C to 310 °C (15 °C /min), 310 °C (2 min)
Injection volume:	1,0 µl, split 1:15
Detection:	MS

[a] DB-35MS (J and W)® 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.

A.5 Capillary electrophoresis (CE)

200 µl of the sample solution (9.6) is mixed with 50 µl HCl (c = 0,01 mol/l) and passed through a membrane filter (0,2 µm). This solution is analysed by means of capillary zone electrophoresis.

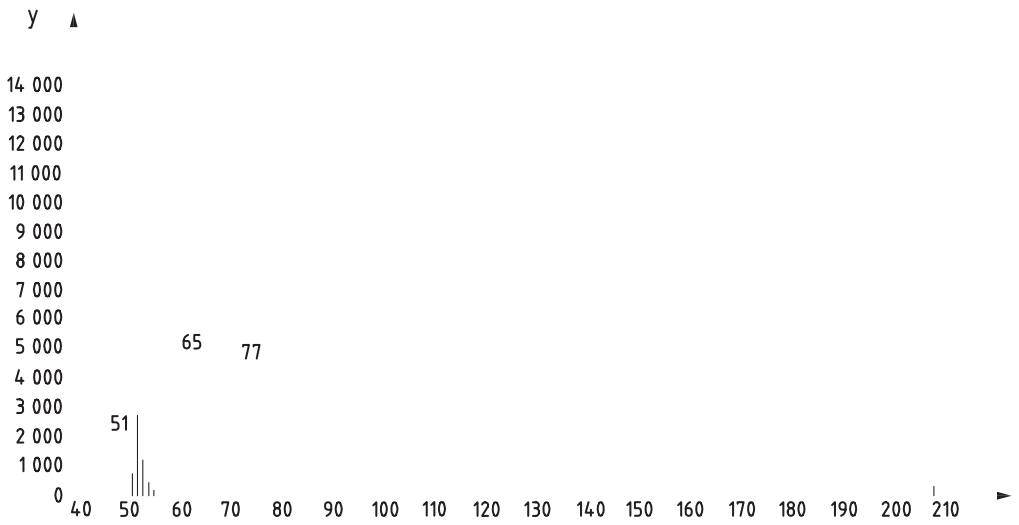
Capillary 1:	56 cm, uncoated, inside diameter 50 µm, with extended light path (Agilent)
Capillary 2:	56 cm, coated with polyvinyl alcohol (PVA), inside diameter 50 µm, with extended light path (Agilent)
Buffer solution:	phosphate buffer solution (c = 50 mmol/l), pH = 2,5
Column temperature:	25 °C
Voltage:	30 kV
Injection time:	4 s
Flushing time:	5 s
Detection:	DAD 214 nm, 254 nm, spectrograph
Quantification:	at 240 nm and 380 nm



Key

1 internal standard
2 4-aminoazobenzene
x time in min
y abundance

Figure A.1 — Total ion current chromatogram of 4-aminoazobenzene with GC-MS

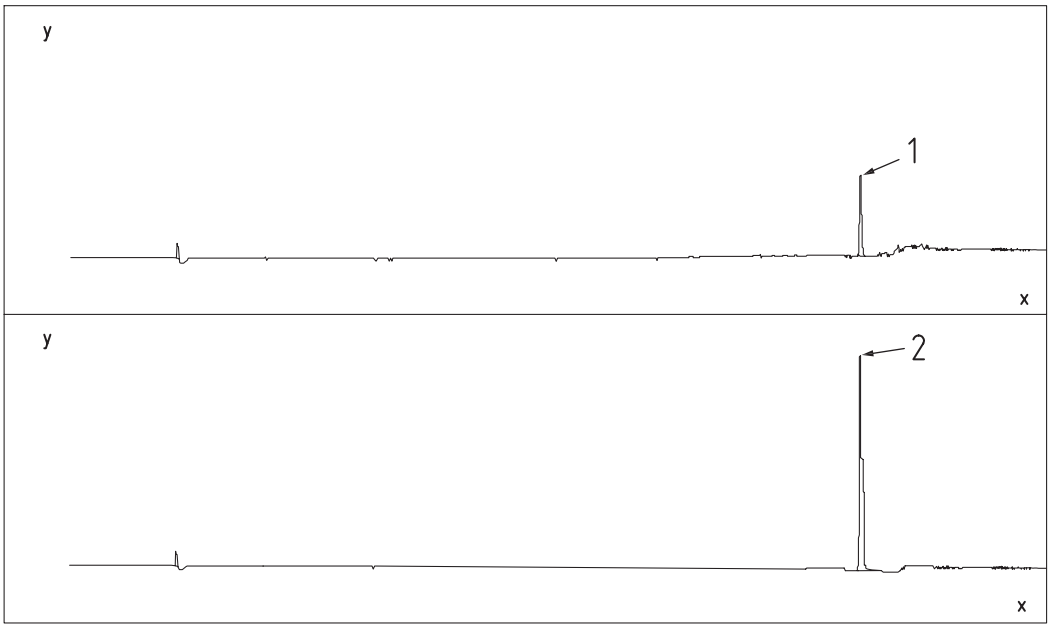


Key

x m/z

y abundance

Figure A.2 — GC-MS 70eV-spectrum of 4-aminoazobenzene



Key

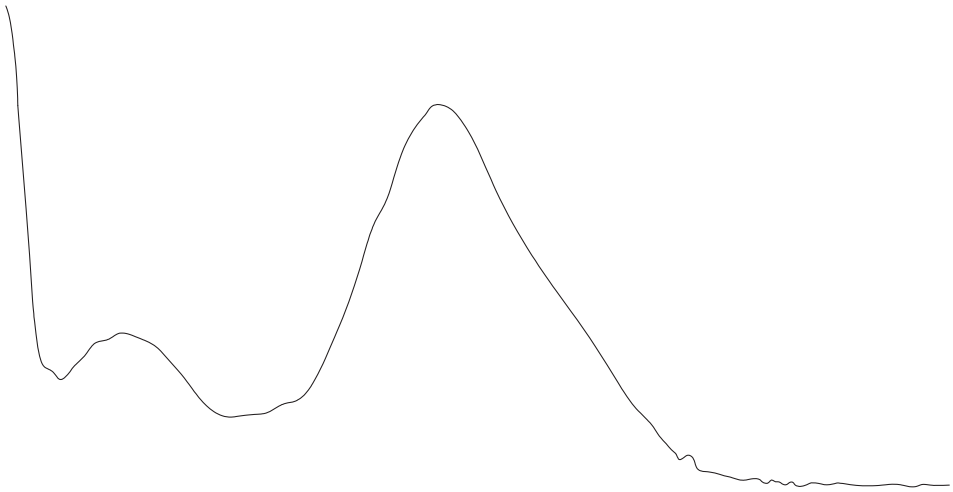
1 240 nm

2 380 nm

x time in min

y absorbance in mAU

Figure A.3 — Chromatogram of 4-aminoazobenzene with HPLC/DAD



Key

x wavelength in nm

y absorbance in mAU

Figure A.4 — HPLC/DAD-spectrum of 4-aminoazobenzene

Annex B
(informative)

Calculation

B.1 General

4-aminoazobenzene levels are calculated from the peak areas. The 4-aminoazobenzene level is calculated as mass portion *w* in mg/kg of the specimen according to one of the following formulae:

B.2 Calibration with internal standard

$$w = \rho_c \times \frac{A_s \times A_{ISC} \times V}{A_c \times A_{ISS} \times m_E} \quad \text{(B.1)}$$

where

w mass portion in mg/kg of 4-aminoazobenzene in the specimen;

ρ_c concentration of the 4-aminoazobenzene in the calibration solution in µg/ml;

A_s peak area of 4-aminoazobenzene in the specimen solution in area units;

A_c peak area of 4-aminoazobenzene 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 9.6 in ml.

m_E weight of the textile specimen, in g.

B.3 Calibration without internal standard

$$w = \rho_c \times \frac{A_s \times V}{A_c \times m_E} \quad \text{(B.2)}$$

where

w mass portion in mg/kg of 4-aminoazobenzene in the specimen;

ρ_c concentration of the 4-aminoazobenzene in the calibration solution in µg/ml;

A_s peak area of 4-aminoazobenzene in the specimen solution in area units;

A_c peak area of 4-aminoazobenzene in the calibration solution in area units;

V final specimen volume made up to according to 9.6 in ml.

m_E weight of the textile specimen, in g.

Annex C
(informative)

Reliability of the method

The data in Table C.1 were obtained in an interlaboratory (ring) test on silk and polyester fabrics.

Table C.1 — Results from method ring test

Parameters	Silk		Polyester	
	GC-MS	HPLC	GC-MS	HPLC
number of participating laboratories	10	11	10	9
number of outliers	0	3	0	1
number of laboratories after elimination of outliers	10	8	10	8
mean value x, mg/kg	77,3	80,7	71,1	52,7
repeatability, r, mg/kg	22,6	11,2	32,6	10,0
standard deviation of the repeatability, s_r, mg/kg	8,1	4,0	11,6	3,6
reproducibility, R, mg/kg	54,7	52,3	54,3	48,2
standard deviation of the reproducibility, s_R, mg/kg	19,6	18,7	19,4	17,2
NOTE Determination of the levels of 4-aminoazobenzene.				

This method was developed by the § 64 LFGB working group "Analysis of proscribed azo colorants" of the German Federal Office of Consumer Protection and Food Safety (BVL) and evaluated in a ring test with 11 participants.

a) Regarding evaluation of repeatability and reproducibility of the ring test results, the following should be considered.

   1) The ring test demonstrated that the ratio of colorant to reducing agent and the age of the reducing agent may have a decisive influence on the quantitative result. Therefore, it is essential to perform the reductive cleavage in strict accordance with the conditions as described in 9.5 (time, temperature and amount particulars).

   2) Another important factor is liquid-liquid extraction, e.g. the separation of aqueous and organic phase to prevent further reaction of the azo bond of 4-aminoazobenzene. Therefore, it is essential to keep conditions as described in 9.6 accurately.

   3) Application of other appropriate internal standards may lead to higher reliability of the GC-MS procedure. This was not considered in the evaluation of the ring test.

b) The silk and the polyester specimen used were especially manufactured for the ring test. For this purpose, the dyeing was processed exclusively with one 4-aminoazobenzene colorant, without use of any other (permitted) azo colorant, i.e. without further substances consuming reducing agent. This way of dyeing should avoid additional influencing factors. Separate tests, however, demonstrated that the addition of other (permitted) azo colorants did not result in a loss of 4-aminoazobenzene.

Annex D
(informative)

Assessment guide — Interpretation of analytical results

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

If the detected amount of 4-aminoazobenzene is more than 30 mg/kg, it shall be assumed that a certain azo colorant was used. Below 30 mg/kg it is at present not possible to make a reliable statement on the use of certain azo colorants 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.

— **In the case of determined levels of 4-aminoazobenzene ≤30 mg/kg**, according to the analysis as carried out, azo colorants which can release 4-aminoazobenzene by reductive cleavage of their azo groups were not detected in the commodity submitted.

— **In the case of determined levels of 4-aminoazobenzene >30 mg/kg**, according to the analysis as carried out, it is suggested that the commodity submitted has been manufactured or treated using azo colorants, which can release 4-aminoazobenzene.

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Price based on 16 pages