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

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Foreword

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

This international standard 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 14362-3:2012 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 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 24362-1 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 part of ISO 24362 is supplementary to ISO 24362-1 and describes a special procedure to detect the use of certain azo colorants in commodities, which may release 4-aminoazobenzene,

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

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

For certain fibre blends both parts of ISO 24362 (without or with extraction) may need to be applied.

The procedure detects as well 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, 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 24362-1:2014, *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*

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

3 General

Certain azo colorants may release, by reductive cleavage of azo group(s), 4-aminoazobenzene, which is proscribed *under 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*.

Table 1 — 4-aminoazobenzene proscribed under Regulation REACH 1907/2006/Annex XVII

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

4 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 the dyes (see ISO 24362-1).

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.

5 Safety precautions

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

Any handling and disposal of this substance 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 aqueous sodium dithionite solution, ρ = 200 mg/ml[1)], freshly prepared, to use immediately after resting for one hour in a closed vessel.

6.2 sodium hydroxide aqueous solution, ω = 2 %[2)]

6.3 n-pentane

6.4 methanol

6.5 chlorobenzene

1) ρ = mass concentration

2) ω = mass fraction (% by weight)

6.6 t-butyl methyl ether

6.7 sodium chloride

6.8 4-aminoazobenzene, with highest available defined purity standard

6.9 internal standards (IS) for gas chromatography, e.g.:

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.

6.10 standard solutions

6.10.1 internal standard solution, prepared by IS (6.9) in *t*-butyl methyl ether, ρ = 10,0 µg/ml

6.10.2 4-aminoazobenzene calibration solution for checking the experimental procedure and preparation of calibration solutions

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

6.11 grade 3 water, complying with ISO 3696.

7 Apparatus

7.1 reaction vessel (20 ml to 50 ml) of heat-resistant glass, with tight closure

7.2 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;

— heating source.

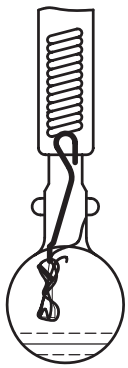


Figure 1 — Apparatus

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

7.3 heating source, capable of maintaining the temperature at (40 ± 2) °C

7.4 centrifuge, capable with a rotation of more than 3 000 r/min

7.5 vacuum rotary evaporator

7.6 pipettes in required sizes or variable pipettes

7.7 ultrasonic bath, capable of ultrasonic power 160 Watt RMS, with controllable heating equipment

7.8 horizontal shaker, capable of frequency of 5 s^{-1}, path length 2 cm to 5 cm

7.9 instrumental equipment

7.9.1 gas chromatography (GC) equipment, with mass selective detector (MS)

7.9.2 high performance liquid chromatography (HPLC) equipment, with gradient elution and diode array detector (DAD) or mass selective detector (MS)

7.9.3 thin layer chromatography (TLC) or high performance thin layer chromatography (HPTLC) equipment, including relevant detection

7.9.4 capillary electrophoresis (CE) equipment, with DAD

NOTE A description of the equipment is given in Annex A.

8 Procedure

8.1 General

Apply this standard to the test specimen that gave a positive result for aniline and 1,4-phenylenediamine or only aniline using ISO 24362-1. Choose 8.3 or 8.4 depending on sample composition.

8.2 Preparation of test specimens

In the case of fabrics with multicoloured patterns, the various colours have to 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 (8.3) cut into strips (if apparatus described in 7.2 is used) or cut into small pieces if other apparatus is used or for specimens to be submitted only to reductive cleavage (8.4).

8.3 Colorant extraction for disperse dyes

8.3.1 Extraction of disperse dyes with chlorobenzene

The textile specimen dyed with disperse dyes (see Annex D from ISO 24362-1) is kept in the extraction apparatus (7.2) for 30 min above 25 ml boiling chlorobenzene. The chlorobenzene extract is allowed to cool down ***to room temperature*** before detaching it from the extraction apparatus.

Concentrate the chlorobenzene extract by the vacuum rotary evaporator (7.5) at a temperature of 45 °C to 60 °C to a small residual quantity. This residue is quantitatively transferred to the reaction vessel with 7 ml methanol (6.4) in total, using an ultrasonic bath to disperse the colorants.

NOTE 1 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.

NOTE 2 For direct determination of a 4-aminoazobenzene-releasing dispersion colorants (e.g. Disperse Yellow 23) an aliquot of this methanolic solution may be immediately used for analysis by LC-DAD-MS.

8.3.2 Textiles only dyed with disperse dyes

Remove the textile specimen from the extraction apparatus (7.2), and discard it if it is completely made of fibres dyed with disperse dyes and/or becomes decolourised after extraction.

8.3.3 Textiles dyed with disperse dyes and/or other dyes

Remove the extracted textile specimen from the extraction apparatus (7.2), if it contains fibres belonging to cases A and/or B (see 8.4 of ISO 24362-1). Remove the solvent by washing the specimen with appropriate solvent e.g. npentane (6.3) or *t*-butyl methyl ether (6.6) 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 dyes (in total 7 ml) for combined reduction.

8.4 Textiles dyed with dyes other than disperse dyes

If the textile specimen contains fibres belonging only to cases A and/or B (see 8.4 of ISO 24362-1) put the test specimen directly in a reaction vessel.

8.5 Reductive cleavage

A quantity of 9 ml of sodium hydroxide solution (6.2) is added to the reaction vessel (8.3.1, 8.3.3 or 8.4). The reaction vessel is tightly closed and shaken vigorously.

Subsequently, 1,0 ml aqueous sodium dithionite solution (6.1) is added for reductive cleavage. 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.

8.6 Separation and concentration of 4-aminoazobenzene

5 ml *t*-butyl methyl ether (6.6) or 5 ml internal standard solution (6.10.1), respectively are added to the reaction solution. Subsequently, 7 g of sodium chloride (6.7) are added and the mixture is shaken in a horizontal way constantly for 45 min; shaking frequency f = 5 s-1.

NOTE 1 The delay time between cooling down and shaking should not exceed 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 7.9.

NOTE 2 For subsequent analysis it may be necessary to change the solvent or to concentrate the extract from 8.5 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.

It is recommended to 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.

NOTE 3 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 specimen is to be kept below −18 °C.

8.7 Calibration solution

8.7.1 Calibration solution for sample preparation without extraction

5 ml *t*-butyl methyl ether (6.6) or 5 ml internal standard solution (6.10.1), respectively are added to 100 µl of the 4-aminoazobenzene calibration solution (6.10.2). This mixture is used for calibration, as the recovery of 4-aminoazobenzene via phase partition according to this procedure is 95 % to 100 %.

8.7.2 Calibration solution for sample preparation with extraction

100 µl of the 4-aminoazobenzene calibration solution (6.10.2) are added to 6,9 ml methanol (6.4), 9 ml sodium hydroxide solution (6.2), 1 ml water, 7 g sodium chloride (6.7) and 5 ml *t*-butyl methyl ether (6.6) or 5 ml internal standard solution (6.10.1), respectively.

This mixture is shaken in a horizontal way constantly for 45 min; shaking frequency f = 5 s-1. For subsequent analysis an aliquot is taken out of the *t*-butyl methyl ether phase. The vial for analysis has to be closed immediately.

8.8 Check of the analytical system

8.8.1 Sample preparation without extraction

To check the procedure, 100 µl of the 4-aminoazobenzene calibration solution (6.10.2) are treated according to 8.5.

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

8.8.2 Sample preparation with extraction

To check the procedure, 100 µl of the 4-aminoazobenzene calibration solution (6.10.2) are added to 6,9 ml methanol. This mixture is treated according to 8.5.

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

8.9 Chromatographic analyses

4-aminoazobenzene detection can be performed using the chromatographic techniques listed in 7.9. 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.

9 Evaluation

9.1 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.

9.2 Reliability of the method

For the reliability of the method see Annex C.

10 Test report

The test report shall 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) results reported as level and detection limit of 4-aminoazobenzene in mg/kg.

NOTE 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 High performance liquid chromatography (HPLC)

As the instrumental equipment of the laboratories may vary (7.9), 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.
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.

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 (A.2.1), reaction time approximately 5 min.

A.2.3

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.

Detection: Successive treatment with reagents 1 and 2 (A.2.1), 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 ® (3,5 µm); (150 × 4,6) mm
Flow rate:	0,6 - 2,0 ml/min (flow gradient, see below)
Column	32 °C;
Injection volume:	5 µl;
Detection:	DAD, spectrograph;
Quantification:	at 240 nm, 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	
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 × 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.

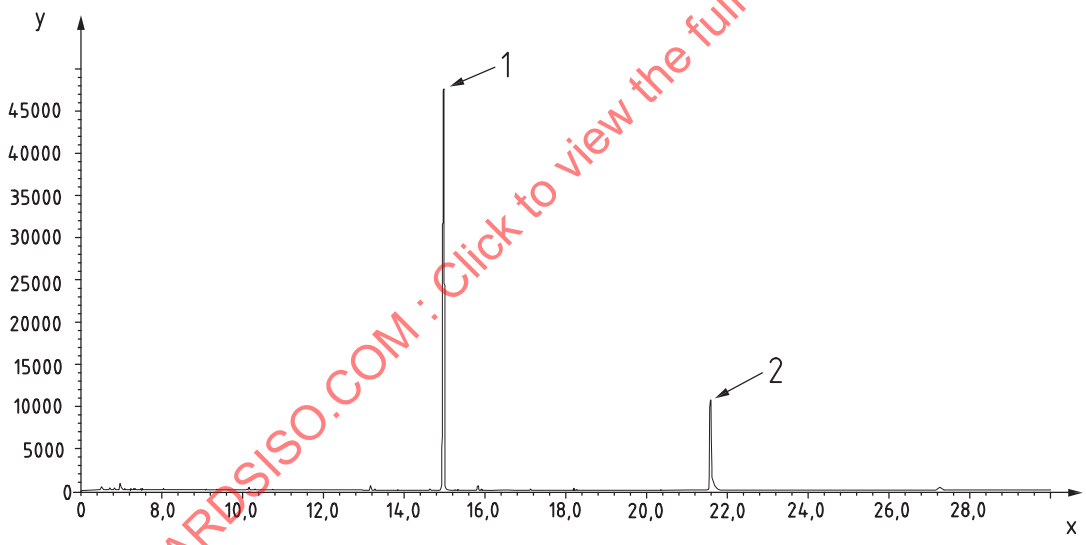
A.4 Capillary gas chromatography/mass selective detector (GC-MS)

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 (8.4) 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.

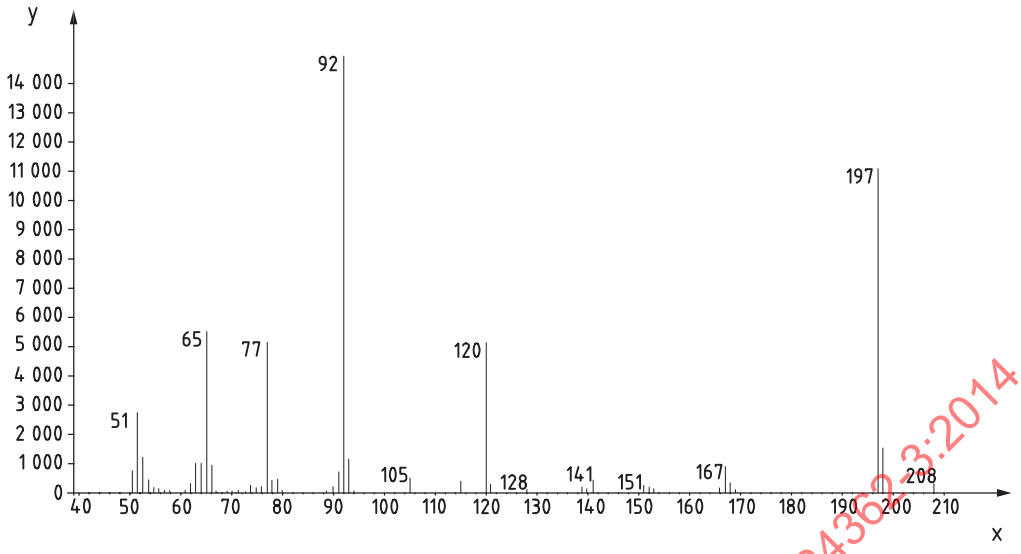
Capillary 1:	56 cm, uncoated, inside diameter 50 µm, with extended light path (Agilent);
Capillary 2:	56 cm, coated with polyvinyl alcohol (PVA), inside diameter 50 µm, with extended light path (Agilent);
Buffer solution:	phosphate buffer solution (c = 50 mmol/l), pH = 2,5;
Column temperature:	25 °C;
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

X time in min
Y abundance
1 internal standard
2 4-aminoazobenzene

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

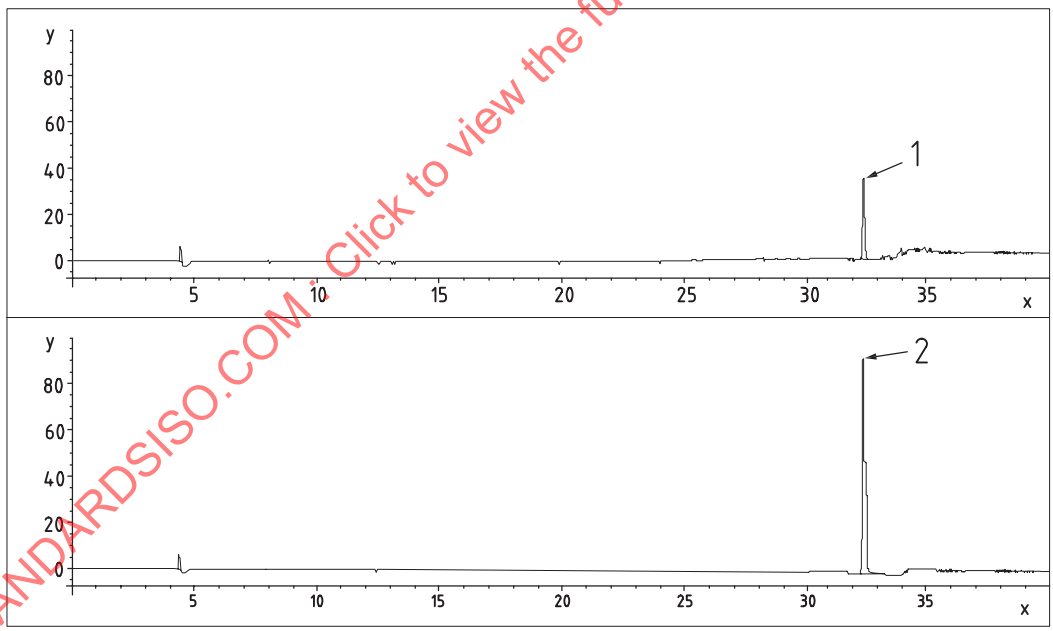


Key

X m/z

Y abundance

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



Key

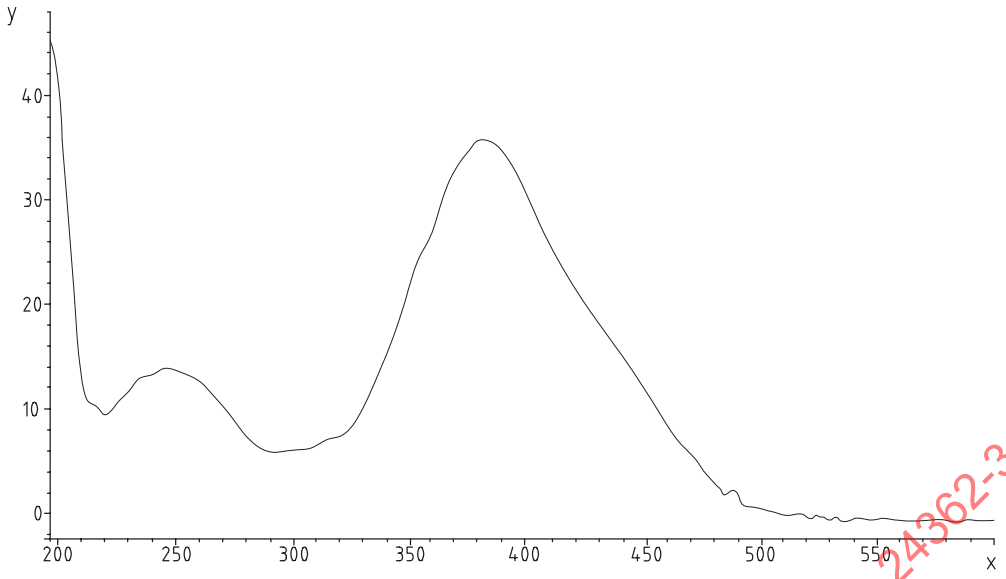
X time in min

Y absorbance in mAU

1 240 nm

2 380 nm

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