BS EN ISO 16373-2:2014

BSI Standards Publication

Textiles — Dyestuffs

Part 2: General method for the determination of extractable dyestuffs including allergenic and carcinogenic dyestuffs (method using pyridine-water) (ISO 16373-2:2014)

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National foreword

This British Standard is the UK implementation of EN ISO 16373-2:2014.

The UK participation in its preparation was entrusted to Technical Committee TCI/80, Chemical testing of textiles.

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

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Textiles - Dyestuffs - Part 2: General method for the determination of extractable dyestuffs including allergenic and carcinogenic dyestuffs (method using pyridine-water) (ISO 16373-2:2014)

Textiles - Colorants - Partie 2: Méthode générale de détermination des colorants extractibles, notamment les colorants allergènes et cancérigènes (méthode utilisant un mélange pyridine/eau) (ISO 16373-2:2014)

Textilien - Farbstoffe - Teil 2: Allgemeines Verfahren zur Bestimmung von extrahierbaren Farbstoffen einschließlich allergener und karzinogener Farbstoffe (Pyridin-Wasser-Verfahren) (ISO 16373-2:2014)

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Foreword

This document (EN ISO 16373-2:2014) has been prepared by Technical Committee CEN/TC 248 “Textiles and textile products” the secretariat of which is held by BSI, in collaboration with Technical Committee ISO/TC 38 "Textiles".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2014, and conflicting national standards shall be withdrawn at the latest by December 2014.

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Endorsement notice

The text of ISO 16373-2:2014 has been approved by CEN as EN ISO 16373-2:2014 without any modification.

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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. www.iso.org/directives

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

ISO 16373-2 was prepared by the European Committee for 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).

ISO 16373 consists of the following parts, under the general title *Textiles — Dyestuffs*:

— *Part 1: General principles of testing coloured textiles for dyestuff identification*

— *Part 2: General method for the determination of extractable dyestuffs including allergenic and carcinogenic (method using pyridine-water)*

— *Part 3: Method for determination of carcinogenic extractable dyestuffs (method using triethylamine/methanol)*

Introduction

The ISO 16373 series deals with dyestuffs used in textile for qualification and quantification.

ISO 16373-1[1)] includes the definition of the dyestuff classes and the description of some procedures to identify qualitatively the dyestuff class used in textile material.

The other parts of ISO 16373 are related to the quantification of some dyestuffs.

In this part of ISO 16373, the principle of the test method is based on the extraction using pyridine-water solution, which has been found to be the most efficient solution to extract a large range of dyestuffs, including allergenic and carcinogenic dyestuffs.

In ISO 16373-3, the principle of the test method is based on extraction using triethylamine/methanol solution. This solution has been found efficient to extract some dyestuffs in some cases.

Additional information related to the recovery rate (to characterize the extraction efficiency) obtained from the application of ISO 16373-3 and this part of ISO 16373 will be summarized in ISO 16373-1.

It is important to note that other test methods exist related to azo dyes, for which a reduction of those extracted azo dyes leads to the release of some aromatic amines, to be detected and determined using chromatography.

The percentage of recovery using the method of this part of ISO 16373 is shown in Annex F for the dyestuff classes (to be defined in ISO 16373-1) acid, basic, direct, disperse, solvent dyestuffs and "mordant dyestuffs" on different textile fibres.

1) To be published.

INTERNATIONAL STANDARD ISO 16373-2:2014(E)

Textiles — Dyestuffs —

Part 2: General method for the determination of extractable dyestuffs including allergenic and carcinogenic dyestuffs (method using pyridine-water)

1 Scope

This part of ISO 16373 specifies the analyses used to detect extractable dyestuffs in textile products, with the extraction performed for all kind of fibres and types of dyestuffs using pyridine/water (1:1). It lists (see Annexes A and B) the allergenic and carcinogenic dyestuffs which can be analysed using this method; the lists of dyestuffs are expandable.

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

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
allergenic dyestuff
dyestuff which may cause an allergic skin reaction

3.2
carcinogenic dyestuff
dyestuff which is classified as carcinogenic substance

Note 1 to entry: Harmonized classification according to the *Globally harmonized system of classification and labelling of chemicals (GHS)*,[2] incorporated in EU Regulation 1272/2008 (CLP)[3].

4 Principle

A coloured test specimen is selected from the textile article and extracted with pyridine/water at 100 °C. The extract is analysed by liquid chromatography/diode array detection (LC/DAD) and/or by liquid chromatography/mass spectrometry (LC/MS).

5 Safety precautions

WARNING —The substances listed in Tables A.1, B.1 and B.2 are classified as substances known to be or suspected to be human allergens or carcinogens.

Ensure that any handling and disposal of these substances is 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.

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

Attention is drawn to any national and local safety regulations.

6 Reagents

Unless otherwise specified, analytical grade chemicals shall be used.

6.1 Pyridine.

6.2 Acetonitrile, chromatographic grade.

6.3 Ammonium acetate.

6.4 Tetrabutylammonium dihydrogen phosphate.

6.5 Deionized water, grade 3 in accordance with ISO 3696.

6.6 Pyridine/water (1:1), prepared by mixing 500 ml pyridine (6.1) and 500 ml water (6.5).

Keep the solution in a brown glass bottle.

6.7 Individual stock solutions, prepared in pyridine-water 1:1, of all reference substances listed in Annexes A and B

It is recommended that reference substances (including those listed in Annexes A and B) of the highest purity grade available on the market be used. The given purity has to be considered for the calculation (see Clause 9).

7 Apparatus

7.1 Apparatus and auxiliaries for sample preparation

7.1.1 Standard laboratory equipment.

7.1.2 Analytical balance, resolution at 0,01 g.

7.1.3 Glass vials (20 ml to 40 ml), with tight closure.

7.1.4 Heating source that generates heat at a temperature of (100 ± 2)°C (thermal block or laboratory sand-bath, controllable).

7.1.5 Autosampler glass vials, with tight closure.

7.1.6 Thermo-sensing device, e.g. thermocouple, to measure at 100 °C with a resolution of 0,1 °C.

7.2 Chromatographic equipment (selected from the following)

7.2.1 Equipment for LC/DAD

— High performance liquid chromatograph (HPLC),

— DAD detector,

— separating column,

— guard column.

7.2.2 Equipment for LC/MS

— High performance liquid chromatograph (HPLC),

— electrospray ion source,

— MS detector,

— separating column,

— guard column.

8 Procedure

8.1 Preparation of test specimen

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

— parts of the textile article;

— nature of the fibre component (fibre composition);

— colours.

Prepare a test specimen of 1,0 g max. by cutting the laboratory sample up into small pieces no larger than 1 cm^2. Determine the mass of the test specimen to the nearest 0,01 g and record it as m_E (see 8.2).

8.2 Extraction

Add 7,5 ml of pyridine/water (1:1) (6.6) to the test specimen and close the vial tightly. Heat the vial in the heating source until the temperature of the solvent reaches (100 ± 2) °C, and hold this temperature for (35 ± 5) min.

Check the time taken for the temperature of the solvent to reach the required temperature using a glass vial (see 8.1) containing 7,5 ml of pyridine/water (1:1)(6.6) with a thermo-sensing element (7.1.6), which is plunged in the solvent and sealed with a septum.

Allow the vial to cool down to 40 °C or below before opening it.

Transfer approximately 1 ml of (test) liquid from the vial to a smaller vial for further analysis.

NOTE This step could be done with a syringe through the closed septum to minimize contact with pyridine.

8.3 Detection and quantification of dyestuff

Dyestuff detection can be performed using the above-specified chromatographic techniques (7.2). If other analytical techniques are used it shall be reported.

Dyestuff quantification is performed by means of HPLC/DAD/MS.

NOTE Some dyes can be quantified with HPLC/DAD.

8.4 Calibration

For the calibration, the mixed standards of the reference substances listed in Table A.1 (and respectively in Tables B.1 and B.2) are prepared in pyridine/water (1:1). The stock solutions are used to prepare mixed standards with concentrations levels of 1 mg/l, 5 mg/l, 10 mg/l and 20 mg/l related to dyestuff content.

9 Calculation and expression of the results

Amounts of dyestuff are usually calculated by means of a software program. The calculation can be carried out manually as described in Annex C.

Amounts of dyestuff are expressed in mg dyestuff per kg of textile (mg/kg).

If the detected amount of a dye is over 100 mg/kg, it is certain that this dye is used – such as one of those listed in Table A.1, B.1 or B.2.

10 Test report

The test report shall refer to this method and shall include at least the following:

a) reference to this part of ISO 16373, i.e. ISO 16373-2;

b) all information necessary for the identification of the test specimen;

c) date of sample receipt and date of analysis;

d) sampling procedure;

e) detection method and quantification method;

f) results reported as level and detection limit per dyestuff, in mg/kg;

g) any deviation from the given procedure.

Annex A
(normative)

List of carcinogenic dyestuffs

See Table A.1.

Table A.1 — Reference carcinogenic dyestuffs

Number[a]	Carcinogenic dyestuff[b]	C. I. Number[c]	CAS Number	Molecular formula
1	Disperse Blue 1	64500	2475–45–8	$C_{14}H_{12}N_4O_2$
2	Solvent Yellow 1 4-aminoazobenzene	11000	60–09–4	$C_{12}H_{11}N_3$
3	Solvent Yellow 2	11020	60–11–7	$C_{14}H_{15}N_3$
4	Solvent Yellow 3 o-aminoazotoluene	11160	97–56–3	$C_{14}H_{15}N_3$
5	Basic Red 9	42500	569–61–9	$C_{19}H_{17}N_3HCl$
6	Basic Violet 14	42500	632–99–5	$C_{20}H_{19}N_3HCl$
7	Disperse Yellow 3	11855	2832–40–8	$C_{15}H_{15}O_2N_3$
8	Acid Red 26	16150	3761–53–3	$C_{18}H_{14}N_2Na_2O_7S_2$
9	Direct Black 38	30235	1937–37–1	$C_{34}H_{25}N_9Na_2O_7S_2$
10	Direct Blue 6	22610	2602–46–2	$C_{32}H_{24}N_6O_{14}S_4Na_4$
11	Direct Red 28	22120	573–58–0	$C_{32}H_{22}N_6Na_2O_6S_2$
12	Disperse Orange 11	60700	82–28–0	$C_{15}H_{11}NO_2$
13	Acid Red 114	23635	6459–9-5	$C_{37}H_{28}N_4Na_2O_{10}S_3$

[a] Numbering used in Tables D.1, D.4, D.5.

[b] Classified according to (GHS)[2] (and to CLP).[3]

[c] Colour index number.[4]

Annex B
(normative)

List of allergenic and other dyestuffs

See Tables B.1 and B.2.

NOTE Not all dyestuffs in Table B.1 are clinically verified as allergenic.

Table B.1 — Reference disperse dyestuffs

Number[a]	Allergenic dyestuff	C.I. Number	CAS Number	Molecular formula
A1	Disperse Blue 1	64500	2475–45–8	$C_{14}H_{12}N_4O_2$
A2	Disperse Blue 3	61505	2475–46–9	$C_{17}H_{16}N_2O_3$
A3	Disperse Blue 7	62500	3179–90–6	$C_{18}H_{18}N_2O_6$
A4	Disperse Blue 26	63305	3860–63–7	$C_{16}H_{14}N_2O_4$
A5	Disperse Blue 35	—	56524–77–7	$C_{15}H_{12}N_2O_4$
A6		—	56524–76–6	$C_{16}H_{14}N_2O_4$
A7	Disperse Blue 102	11945	12222–97–8	$C_{15}H_{19}N_5O_4S$
A8	Disperse Blue 106	111935	12223–01–7	$C_{14}H_{17}N_5O_3S$
A9	Disperse Blue 124	111938	61951–51–7	$C_{16}H_{19}N_5O_4S$
A10	Disperse Brown 1	11152	23355–64–8	$C_{16}H_{15}N_4O_4Cl_3$
A11	Disperse Orange 1	11080	2581–69–3	$C_{18}H_{14}N_4O_2$
A12	Disperse Orange 3	11005	730–40–5	$C_{12}H_{10}N_4O_2$
A13	Disperse Orange 37/76/59	11132	13301–61–6	$C_{17}H_{15}N_5O_2Cl_2$
A14	Disperse Red 1	11110	2872–52–8	$C_{16}H_{18}N_4O_3$
A15	Disperse Red 11	62015	2872–48–2	$C_{15}H_{12}N_2O_3$
A16	Disperse Red 17	11210	3179–89–3	$C_{17}H_{20}N_4O_4$
A17	Disperse Yellow 1	10345	119–15–3	$C_{12}H_9N_3O_5$
A18	Disperse Yellow 3	11855	2832–40–8	$C_{15}H_{15}N_3O_2$
A19	Disperse Yellow 9	10375	6373–73–5	$C_{12}H_{10}N_4O_4$
A20	Disperse Yellow 39	480095	12236–29–2	$C_{17}H_{16}N_2O$
A21	Disperse Yellow 49	—	54824–37–2	$C_{22}H_{22}N_4O_2$

[a] Numbering used in Tables D.2 and D.3.

Table B.2 — Other reference dyestuffs

Number[a]	Other dyestuff	CI Number	CAS Number	Molecular formula
O1	Disperse Yellow 23	26070	6250–22–3	$C_{18}H_{14}N_4O$
O_2	Disperse Orange 149	—	85136–74–9	$C_{25}H_{26}N_6O_3$
O3	Navy Blue 018112	—	118685–33–9	$C_{39}H_{23}ClCrN_7O_{12}\ S\ Na_2$
		—	—	$C_{46}H_{30}CrN_{10}O_{20}\ S_2\ Na_3$
O4	Disperse Orange 61	111355	55281–26–0	$C_{17}H_{15}Br_2N_5O_2$

[a] Numbering used in Tables D.2 and D.3.

Annex C
(normative)

Calculation

Dyestuff levels are calculated from the peak areas of the individual dyestuff components.

The dyestuff level is calculated as a mass fraction, w, in mg/kg, of the extractable dyestuff in the test solution, using Formula (1):

$$w = \frac{\rho_c \times V}{m_E} \tag{C.1}$$

where

w is the concentration of dyestuff, in mg/kg;

ρ_c is the concentration of the dyestuff in the test solution, in mg/l (from the calibration curve);

V is the volume (final specimen volume), in ml;

m_E is the mass of the textile specimen, in g.

Annex D
(informative)

Examples of chromatographic methods

D.1 High performance liquid chromatography (HPLC/DAD) method for disperse and carcinogenic dyestuffs

D.1.1 UHPLC system with pressure over 400 bar[2)]

Eluent 1:	2 % Tetrabutylammonium dihydrogen phosphate with 10 % acetonitrile		
Eluent 2:	Acetonitrile		
Stationary phase:	HALO-C18 2,7 µm; 150 × 2,1 mm with guard column		
Flow rate:	0,5 ml/min		
Column temperature:	40 °C		
Injection volume:	5 µl		
Pressure:	470 bar/6 800 psi		
Detection:	DAD, spectrograph		
Quantification:	at 400 nm, 500 nm, 600 nm, (700 nm)		
Gradient:	Time [min]:	Eluent 2 [%]:	Flow [ml]:
	1,0	0	0,5
	15,0	47	0,5
	25,0	55	0,5
	26,0	100	0,666
	28,0	100	0,8
	30,0	100	0,8
	35	0	0,5

D.1.2 Standard HPLC system with maximum pressure of 400 bar

Conditions as in D.1.1, with the exception of the following parameters:

Stationary phase:	HALO-C18 2,7 µm; 100 × 2,1 mm with guard column
Column temperature:	50 °C

2) 1 bar = 0,1 MPa = 0,1 N/mm^2 = 10^5 N/m^2.

Pressure:	250 bar/3 600 psi			
Gradient:	Time [min]:	Eluent 2 [%]:	Flow [ml]:	
	1,0	0	0,5	
	15,0	47	0,5	
	30,0	80	0,5	
	35,0	80	0,5	start flow gradient
	40,0	80	flow gradient from 0,5 to 1,2 ml/min	
	45,0	100	1,2	end flow gradient
	46,0	100	1,2	
	46,1	0	0,5	
	53,0	0	0,5	

Table D.1 — Reference substances (carcinogenic dyestuffs) and HPLC/DAD retention times

Number	Carcinogenic dyestuff	CAS Number	Retention time [min] with UHPLC (see D.1.1)	Quantification wavelength		
				400 nm	500 nm	600 nm
1	Disperse Blue 1	2475–45–8	8,8			X
2	Solvent Yellow 1 4-aminoazobenzene	60–09–4	14,6	X		
3	Solvent Yellow 2	60–11–7	21,2	X		
4	Solvent Yellow 3 o-aminoazotoluene	97–56–3	18,2	X		
5	Basic Red 9	569–61–9	1,9		X	
6	Basic Violet 14	632–99–5	6,5		X	
7	Disperse Yellow 3	2832–40–8	15,8	X		
8	Acid Red 26	3761–53–3	15,1		X	
9	Direct Black 38	1937–37–1	17,7 18,7			X
10	Direct Blue 6	2602–46–2	16,8			X
11	Direct Red 28	573–58–0	16,0		X	
12	Disperse Orange 11	82–28–0	15,6		X	
13	Acid Red 114	6459–9-5	24,6		X	

NOTE All standards of Tables D.1 and D.2 were diluted in methanol and as well in pyridine/water 1:1. Comparing the two solutions with HPLC/DAD, no differences in retention time as well in DAD spectra occurred.

Table D.2 — Reference substances (allergenic and other dyestuffs) and HPLC/DAD retention times

Number	Allergenic or other dyestuff	CAS Number	Retention time [min] with UHPLC (see D.1.1)	Quantification wavelength			
				400 nm	500 nm	600 nm	700 nm
A1	Disperse Blue 1	2475–45–8	8,8			X	
A2	Disperse Blue 3	2475–46–9	12,2			X	
A3	Disperse Blue 7	3179–90–6	11,3 12,4 14,7			X	
A4	Disperse Blue 26	3860–63–7	16,8			X	
A5	Disperse Blue 35	56524–77–7	17,4			X	
A6	Disperse Blue 35	56524–76–6	22,2				X
A7	Disperse Blue 102	12222–97–8	13,7			X	
A8	Disperse Blue 106	12223–01–7	15,2			X	
A9	Disperse Blue 124	61951–51–7	18,9			X	
A10	Disperse Brown 1	23355–64–8	16,6		X		
A11	Disperse Orange 1	2581–69–3	25,3	(X)	X		
A12	Disperse Orange 3	730–40–5	15,5	X	(X)		
A13	Disperse Orange 37/76	13301–61–6	23,1	X	(X)		
A14	Disperse Red 1	2872–52–8	17,2		X		
A15	Disperse Red 11	2872–48–2	12,0		X	X	
A16	Disperse Red 17	3179–89–3	14,7 15,0		X		
A17	Disperse Yellow 1	119–15–3	13,6	X			
A18	Disperse Yellow 3	2832–40–8	15,8	X			
A19	Disperse Yellow 9	6373–73–5	13,6	X			
A20	Disperse Yellow 39	12236–29–2	15,1 16,1	X			
A21	Disperse Yellow 49	54824–37–2	19,035	X			
O1	Disperse Yellow 23	6250–22–3	24,0	X			
O2	Disperse Orange 149	85136–74–9	27,5		X		
O3	Navy Blue 018112	118685–33–9	21,6 27,0			X	
O4	Disperse Orange 61	55281–26–0	24,1	X	X		

NOTE 1 All standards of Tables D.1 and D.2 were diluted in methanol and as well in pyridine/water 1:1. Comparing the two solutions with HPLC/DAD, no differences in retention time as well in DAD spectra occurred.

NOTE 2 With this HPLC/DAD method, it is possible to identify Disperse Orange 37/76 if Disperse Orange 61 is present. It is also possible to identify Disperse Orange 3 if Disperse Yellow 3 is present.

D.2 HPLC/DAD/MS method for disperse dyestuffs

D.2.1 Chromatographic conditions for the HPLC/DAD/MS

Eluent 1:	Ammonium acetate 10 mmol pH 3,6		
Eluent 2:	Acetonitrile		
Stationary phase:	XDB-C18 3,5 µm; 100 × 2,1 mm with guard column		
Flow rate:	0,3 ml/min		
Column temperature:	35 °C		
Injection volume:	5 µl		
Pressure:	120 bar		
Detection:	DAD, spectrograph MS		
Gradient:	Time [min]:	Eluent 2 [%]:	Flow [ml]:
	0	40	0,3
	5	60	0,3
	7,5	85	0,3
	9	98	0,3
	13	40	0,3
Runtime	15 min		
Post time	3 min		

D.2.2 Device parameters for the HPLC/DAD/MS method

DAD detection scan 210 nm to 800 nm

MS detection scan 100 amu to 1 000 amu

MS ionization electrospray, positive, negative CID 80V

D.2.3 Measurement parameters for the HPLC/DAD/MS method

The dispersion dyestuffs listed in Table D.3 are separated in the liquid chromatograph and identified with UV/VIS and MS detection methods. The retention times, the visual and mass spectrometer data are listed in Table D.3. The mass measurement value with the greatest signal intensity value in the component's mass spectrum are used for quantification.

Table D.3 — Reference substances and HPLC MS retention times

Number	Dyestuff	CAS Number	Retention time	VIS signal nm max	UV signal nm max	ESI positive m/z signal	ESI negative m/z signal
A1	Disperse Blue 1	2475–45–8	2,0	620	240	268/269	
A2	Disperse Blue 3	2475–46–9	2,3	636	260	297	
A3	Disperse Blue 7	3179–90–6	3,7	668	242	359	
A4	Disperse Blue 26	3860–63–7	11,2	665	240	299	
A5	Disperse Blue 35	56524–77–7	9,7	648	240	285	
A6		56524–76–6	11,6	680	239	299	
A7	Disperse Blue 102	12222–97–8	4,8	616	292	366	
A8	Disperse Blue 106	12223–01–7	6,9	614	292	336	
A9	Disperse Blue 124	61951–51–7	10,0	598	292	378	
A10	Disperse Brown 1	23355–64–8	8,1	445	250	433	
A11	Disperse Orange 1	2581–69–3	11,6	466	276	319/320	
A12	Disperse Orange 3	730–40–5	7,9	434	276	243	
A13	Disperse Orange 37/76	13301–61–6	11,2	430	268	392/394	
A14	Disperse Red 1	2872–52–8	8,9	496	290	315	
A15	Disperse Red 11	2872–48–2	3,9	532	257	269	
A16	Disperse Red 17	3179–89–3	6,0	504	294	345	
A17	Disperse Yellow 1	119–15–3	5,8	366	264	276	274
A18	Disperse Yellow 3	2832–40–8	7,7	356	250	270	
A19	Disperse Yellow 9	6373–73–5	5,6	368	240	275	273
A20	Disperse Yellow 39	12236–29–2	7,4/8,4	368	284	291	
A21	Disperse Yellow 49	54824–37–2	10,1	446	234	375	
O1	Disperse Yellow 23	6250–22–3	11,4	383	235	303	301
O2	Disperse Orange 149	85136–74–9	12,6	455	265	459/476	

D.2.4 Calibration and evaluation with the HPLC/DAD/MS method

For calibration stock solutions of the reference substances mentioned in Table D.3 are prepared in pyridine-water 1:1. The stock solutions are used to prepare mixed standard solutions with single substance concentration levels of 1 mg/l, 2,5 mg/l, 5 mg/l, 10 mg/l, 20 mg/l related to the dyestuff content.

The test is carried out and the mass spectrometer data are used for quantitative evaluation. Using the masses given in Table D.3, a calibration curve is plotted between the MS signal area and the standard concentration.

D.2.5 Evaluation with the HPLC/DAD/MS method

A quantification using just the HPLC/DAD method is only permitted with a complete chromatographic separation of the target compounds.

NOTE 1 It is not possible to identify Disperse Orange 37/76/59 with 100 % certainty with the HPLC/DAD method if Disperse Orange 61 is present.

NOTE 2 It is not possible to quantify Disperse yellow 3 and Disperse orange 3 with HPLC-DAD if both substances are present in the solution. MS-quantification is necessary.

D.2.6 Accuracy of the HPLC/DAD/MS method

The data was determined in 10 replicate experiments with real samples and standard solutions. A standard deviation of 9,54 % resulted for the whole process that is preparing the sample and determination with a mass spectrometer.

D.2.7 Detection limit of the HPLC/DAD/MS

Reference substance disperse blue 1 was chosen to calculate the detection limit and the determination limit, as it showed the lowest signal intensity and thus determined the range of application for the method.

To perform the calculation, 5 mix standards with concentrations 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l, and 10 mg/l were made. The readings were taken and a calibration curve plotted between the MS signal area and the standard concentration. The decision limit was determined as 0,7 mg/l and the determination limit as 2,41 mg/l for the reference substance. The determination limit can be lowered further by SIM or increasing the concentration of the sample solution.

D.3 HPLC/DAD/MS method for carcinogenic dyestuffs (method example 1)

D.3.1 Chromatographic conditions of the HPLC/DAD/MS method for carcinogenic dyestuffs and devices for HPLC/DAD/MS technique

Eluent 1:	Ammonium acetate 10 mmol pH 3,6
Eluent 2:	Acetonitrile
Stationary phase:	XDB-C18 3,5 µm; 100 × 2,1 mm with guard column
Flow rate:	0,300 ml/min
Column temperature:	35 °C
Injection volume:	5 µl
Pressure:	max 200 bar
Detection:	DAD, spectrograph MS

Gradient:	Time [min]:	Eluent 2 [%]:	Flow [ml]:
	0	40	0,3
	5	60	0,3
	7,5	85	0,3
	9	98	0,3
	13	40	0,3
Runtime	15 min		
Post time	3 min		

D.3.2 Device parameters for HPLC/DAD/MS technique

DAD-Detection scan 210 nm to 800 nm

MS-Detection: SIM-Method (ESI positive/ESI negative; m/z-Signals, see Table D.4)

MS ionization electro spray, positive/negative CID voltage at 80V

D.3.3 Measurement parameters for HPLC/DAD/MS technique

The carcinogenic dyestuffs mentioned in Table D.4 are separated by liquid chromatography and identified by UV/VIS- and MS-detection. The retention times, the optical and mass spectrometric data are mentioned in Table D.4. The quantification is conducted by using the M^+/M—mass value with the highest signal intensity value.

Table D.4 — Reference substances and HPLC-MS retention times, parameters electrospray positive and negative MS-, VIS- and UV-Data

Number	Carcinogenic dyestuff	CAS Number	Retention time	VIS signal nm max	ESI pos. m/z signal	ESI neg. m/z signal
1	Disperse Blue 1	2475–45–8	1,9	620	**268/269**	
2	Solvent Yellow 1	60–09–4	7,0	384	**198**/*199*	
3	Solvent Yellow 2	60–11–7	10,5	413	**226**/*227/228*	
4	Solvent Yellow 3	97–56–3	9,5	388	**226**/*227/228*	
5	Basic Red 9	569–61–9	1,4	540	**288**/*289/290*	
6	Basic Violet 14	632–99–5	1,6	550	**302**/*303/304*	
7	Disperse Yellow 3	2832–40–8	7,8	352	**270**/*271/272*	
8	Acid Red 26	3761–53–3	0,96/1,2	512	**437**/*438*	**435**
9	Direct Black 38	1937–37–1	1,9	600	*724*/**738**	*722*/**736**
10	Direct Blue 6	2602–46–2	0,96	592		**421/442**
11	Direct Red 28	573–58–0	1,36	510		**325/651**
12	Disperse Orange 11	82–28–0	7,9	480	**238**/*239/240*	

D.3.4 Calibration and evaluation with the HPLC/DAD/MS method

For calibration stock solutions of the reference substances mentioned in Table D.4 are prepared in pyridine/water (1:1). The stock solutions are used to prepare mixed standard solutions with concentration levels of 1 mg/l, 5 mg/l, 10 mg/l and 20 mg/l related to dyestuff content in the (solid) reference substances. The measurement is conducted as described in 8.3 and mass spectrometric data are used for the quantitative evaluation. Referring to the masses given in Table D.3 a calibration curve between MS signal units and reference concentrations is constructed.

D.3.5 Evaluation with the HPLC/DAD/MS method

A quantification using just the HPLC/DAD method is only permitted with a complete chromatographic separation of the target compounds.

D.3.6 Accuracy of the HPLC/DAD/MS method

The data were determined from 10 replicate experiments with real samples and standard solutions.

Evaluation of the accuracy (repeatability) of the method resulted in a relative standard deviation of 7 % for the whole process consisting of sample preparation and determination with a mass spectrometer.

D.3.7 Detection limit of the HPLC/DAD/MS method

To calculate the detection and quantification limits reference substance number 10 has been chosen as this substance shows the lowest signal intensity and therefore determines the operating conditions of the method.

For the determination according to DIN 32645[5], 10 composite standards with concentration levels ranging from 0,1 mg/l up to 10 mg/l were prepared. Testing was conducted as described in 8.3 and calibration curves constructed referring to MS-signals and standard concentration levels. For reference substance 10, a detection limit of 1,7 mg/l and a quantification limit of 2,5 mg/l were calculated. The quantification level can be decreased by further reduction of the sample solution.

D.4 HPLC/DAD/MS method for carcinogenic dyestuffs (Method example 2)

D.4.1 Chromatographic conditions of the HPLC/DAD/MS for method carcinogenic dyestuffs and devices for HPLC/DAD/MS technique

Eluent 1:	Ammonium acetate 10 mmol pH 3,6		
Eluent 2:	Acetonitrile		
Stationary phase:	Synergy polar-RP 80A (Silica) 4 µm; 150 × 2,0 mm with guard column (2 × 4 mm, polar RP)		
Flow rate:	0,300 ml/min		
Column temperature:	35 °C		
Injection volume:	5 µl		
Pressure:	max 200 bar		
Detection:	DAD, spectrograph		
	MS		
Method A with gradient	Time [min]:	Eluent 2 [%]:	Flow [ml]:
	0	98	0,3
	2	60	0,3
	4,5	20	0,3
	7,5	20	0,3
Method B isocratic		70	0,3
Runtime	8 min		
Post time	4 min		

D.4.2 Device parameters for HPLC/DAD/MS technique

DAD-Detection scan 210 nm–800 nm

MS-Detection: SIM-Method (ESI positive/ESI negative; m/z-signal, see Table D.4)

MS-Ionization-Electrospray, positive/negative, CID voltage at 80V.

See Table D.5.

Table D.5 — Reference substances and HPLC-MS retention times

Number	Carcinogenic dyestuff	Method A with gradient HPLC-MS retention time (min) Silica-based phase	Method B isocratic HPLC-MS retention time (min) Silica-based phase
1	Disperse Blue 1	1,56	1,8
2	Solvent Yellow 1	1,6	2,2
3	Solvent Yellow 2	1,86	3,2
4	Solvent Yellow 3	1,7	2,6
5	Basic Red 9	4,9	2,1
6	Basic Violet 14	4,7	2,2
7	Disperse Yellow 3	1,6	2,1
8	Acid Red 26	0,92	1,0
9	Direct Black 38	0,99	1,1
10	Direct Blue 6	1,26	0,94
11	Direct Red 28	0,93	1,0
12	Disperse Orange 11	1,7	2,49

Calibration and evaluation are conducted according to D.3.4.

Annex E
(informative)

Reliability of the method

Annex E presents a summary of round robin tests performed in 2008 by working group, NA 062-05-12 AA, *Textilchemische Prüfverfahren und Fasertrennung*, of DIN, Germany, showing the reproducibility standard deviation (RSD).

Cotton	**Number of trials**		**Mean**		**RSD**	
RV003	DAD	MS	DAD	MS	DAD	MS
	n	n	mg/kg	mg/kg	%	%
Direct red 28	4	7	298	232	17	20
Direct black 38	5	7	110	111	14	12

Polyester	**Results n**		**Mean**		**RSD**	
RV004	DAD	MS	DAD	MS	DAD	MS
	n	n	mg/kg	mg/kg	%	%
Disperse yellow 3	5	6	219	245	39	37
Disperse orange11	5	6	211	207	14	20

Polyester	**Results n**		**Mean**		**RSD**	
RV004	DAD	MS	DAD	MS	DAD	MS
	n	n	mg/kg	mg/kg	%	%
Disperse yellow 3	5	6	219	245	39	37
Disperse orange11	5	6	211	207	14	20

Polyester	**Results n**		**Mean**		**RSD**	
RV005	DAD	MS	DAD	MS	DAD	MS
	n	n	mg/kg	mg/kg	%	%
Solvent yellow 1	5	7	220	215	21	19
Solvent yellow 2	5	7	102	99	31	34
Solvent yellow 3	5	6	167	155	34	29

Wool/Polyacrylic(50:50)	**Results n**		**Mean**		**RSD**	
RV006	DAD	MS	DAD	MS	DAD	MS
	n	n	mg/kg	mg/kg	%	%
Acid red 26	5	6	226	186	9	16
Basic violet 14	2	7	114	112	35	24

Six laboratories took part in the round robin; one laboratory performed the whole experiment twice.

Sample preparation was done in each laboratory six times.

Annex F
(informative)

Multiple-extraction of several textile fibres with pyridine/water

F.1 General

The majority of dyed textiles were prepared by DyStar Colours Distribution GmbH, Leverkusen for the DIN-working group NA 062-05-12 AA, *Textilchemische Prüfverfahren und Fasertrennung*, Germany.

A single material was provided by the Institute for Interlaboratory Studies (IIS), Netherlands, and has been used before in a round robin test (Material #0821 Round robin test March 2008). This material is dyed with Disperse blue 1 (B1) and Disperse orange 3 (O3). The dyestuff content is unknown.

All materials dyed with Acid red 114 dyed materials were produced for a Japanese round robin test.

The following dyestuffs were provided by Bureau Veritas, Schwerin: Acid red 26 (AR26), Basic violet 14 (BV 14), (Basic red 9 (BR9)), Direct black 38 (DBk38), Direct red 28 (DR28), Disperse yellow 3 (Y3), Disperse orange 11 (O11), Solvent yellow 1, 2 and 3 (SY1, SY2, SY3).

Direct blue 6 (DB6) was provided by TÜV Rheinland LGA Products, Cologne.

The extractions were done at TÜV Rheinland LGA Products (TRLP) GmbH and CITEVE Vila Nova de Famalicão, Portugal.

Table F.1 lists the recovery R after the first extraction step in relation to total recovery after 2-4 extraction steps. After these extraction steps the textiles are colourless.

The dyeing liquor was calculated to the theoretical dyestuff content in the fabric, which is listed in brackets in mg/kg. In reality it is not possible to get these contents, because quite relevant amounts of dyestuff will be lost in the dyeing process.

F.2 Discussion of results

Interestingly, although the extraction of the fibres has to be repeated two to four times to get a colourless textile, in the majority of cases the recovery after the first extraction is nearly 100 %.

A possible explanation for this observation is that already in the 1st extraction step the total dyestuff is dissolved and no longer fixed on the fibre. The small amount of solvent remaining on the fibre after the 1st step contains the dyestuff molecules which are diluted during the following extraction steps.

The basic dyestuffs on polyacrylic fibres make an exception, presumably because of cationic binding. Usually it is difficult to decolourize polyacrylic fibres. Nevertheless, in these experiments nearly 50 % dissolution was achieved in the first extraction step.

Summary: The results demonstrate that pyridine/water is a suitable solvent for the extraction of the investigated carcinogenic dyestuffs.

For the majority of textile materials examined here, about 100 % of the total dyestuff content is obtained in the first extraction step.

Even for difficult materials like polyacrylic fibres recoveries are in the range of 50 %.

Table F.1 — Recovery, R, after first extraction step in relation to total recovery after 2–4 extraction steps

Dyestuff	Fibre													
	Cotton		Polyester		Polyester/cotton 50:50		Polyamide		Polyacrylic		Wool		Wool/polyacrylic 50:50	
	R	c	R	c	R	c	R	c	R	c	R	c	R	c
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Acid red 26	—	—	—	—	—	—	104	(350)	—	—			104	(250)
Acid red 114	—	—	—	—	—	—	87*	(10 000)	—	—	83*	(2 000)	—	—
Basic violet 14	—	—	—	—	—	—	—	—	44	(300)	—	—	53	(200)
Basic red 9	—	—	—	—	—	—	—	—	41	(200)	—	—	—	—
Direct black 38	103	(300)	—	—	103	(200)	—	—	—	—	—	—	—	—
Direct blue 6	104	(280)	—	—	—	—	—	—	—	—	—	—	—	—
Direct red 28	102	(400)	—	—	107	(250)	—	—	—	—	—	—	—	—
Disperse blue 1	—	—	108	(unknown)		—	—	—	—	—	—	—	—	—
Disperse orange 3	—	—	85	(unknown)	—	—	—	—	—	—	—	—	—	—
Disperse orange 11	—	—	93	(250)	—	—	—	—	—	—	—	—	—	—
Disperse yellow 3	—	—	95	(300)	98	(200)	—	—	—	—	—	—	—	—
Solvent yellow 1	—	—	102	(300)	—	—	—	—	—	—	—	—	—	—
Solvent yellow 2	—	—	98	(150)	—	—	—	—	—	—	—	—	—	—
Solvent yellow 3	—	—	101	(250)	100	(300)	—	—	—	—	—	—	—	—

The theoretical dyestuff concentration, c, in the fabric is given in parentheses/brackets.

The extractions were done at TÜV Rheinland LGA Products (TRLP) GmbH and, where indicated by an asterisk (*), at CITEVE Vila Nova de Famalicão, Portugal.

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