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Safety of toys —

Part 7:
Requirements and test methods for finger paints

Sécurité des jouets —

Partie 7: Exigences et méthodes d'essai pour les peintures digitales

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Contents

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).

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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

The committee responsible for this document is ISO/TC 181, *Safety of toys*.

ISO 8124 consists of the following parts, under the general title *Safety of toys*:

- *Part 1: Safety aspects related to mechanical and physical properties*
- *Part 2: Flammability*
- *Part 3: Migration of certain elements*
- *Part 4: Swings, slides and similar activity toys for indoor and outdoor family domestic use*
- *Part 5: Determination of total concentration of certain elements of toys*
- *Part 6: Certain phthalate esters in toys and children's products*
- *Part 7: Requirements and test methods for finger paints*
- *Part 8: Age determination guidelines*

Introduction

This part of ISO 8124 contains requirements which are intended to reduce the risks to children when finger paints are used as intended or in a foreseeable way, bearing in mind the behaviour of children. In particular it is recognized that finger paints present different risks when compared to other toys because of their intended use which requires application to the hands and fingers of young children. It cannot be ruled out that some of the paint material will be ingested or flicked into the eye or that prolonged contact with the skin will occur. Therefore, in order to address the particular risks associated with finger paints, this part of ISO 8124 contains requirements relating to the ingredients that may be used in the manufacture of finger paints and limitations on certain impurities that may be found in these ingredients. It also sets out certain requirements on the packaging and labelling of finger paints.

Chemical Abstract Service Registry Numbers (CAS) or Colour Index Numbers given in the Tables are provided for information purposes only.

Annex A provides rationale for the requirements.

Safety of toys —

Part 7:
Requirements and test methods for finger paints

1 Scope

This part of ISO 8124 specifies requirements for the substances and materials used in finger paints. It is applicable to finger paints only.

It is not applicable to paints intended to be applied to the face or body e.g. face paints.

Additional requirements are specified for markings, labelling and containers.

2 Normative references

The following referenced documents are indispensable for the application 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 787-9, *General methods of test for pigments and extenders — Part 9: Determination of pH value of an aqueous suspension*

ISO 8124-3:2010, *Safety of toys — Part 3: Migration of certain elements*

3 Terms and definitions

For the purposes of this document the following definitions apply.

3.1
finger paint
aqueous semi-solid or liquid, coloured mixture specially designed for children to apply directly to suitable surfaces with the fingers and hands

Note 1 to entry: Finger paint supplied in powder form is mixed with water according to the manufacturer's instructions before being considered in relation to this part of ISO 8124.

3.2
colourant
pigment or dyestuff

Note 1 to entry: For definitions of pigment and dyestuff see A.2.

3.3
extender
material in granular or powder form, insoluble in the medium and used to modify or influence certain physical properties

Note 1 to entry: [SOURCE: ISO 4618:2014, definition 2.102, modified — Note 1 to entry deleted]

3.4
humectant
substance that delays the drying process

3.5
binding agent
water-soluble or miscible, non-volatile component that binds the paint to the surface to which it has been applied

3.6
preservative
substance that prevents the growth of undesirable micro-organisms

3.7
surfactant
surface active substance that reduces the surface tension of the solution

Note 1 to entry: It can be used to make components miscible.

3.8
embittering agent
substance that gives the product a bitter taste

4 Requirements

4.1 General

Finger paints supplied in powder form shall be assessed against this part of ISO 8124 when mixed with water in accordance with the manufacturer’s instructions.

When assessed according to the rules set out in the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), a finger paint shall not be classified with any of the following health hazards:

— acute toxicity (hazard class 3.1);

— skin corrosion/irritation (hazard class 3.2);

— serious eye damage/eye irritation (hazard class 3.3);

— respiratory or skin sensitization (hazard class 3.4);

— germ cell mutagenicity (hazard class 3.5);

— carcinogenicity (hazard class 3.6);

— reproductive toxicity – adverse effects on sexual function and fertility or on development (hazard class 3.7);

— specific target organ toxicity — single exposure effects other than narcotic effects (hazard class 3.8);

— specific target organ toxicity — repeated exposure (hazard class 3.9);

— aspiration hazard (hazard class 3.10).

4.2 Colourants

See A.2.

4.2.1 Colourants used in finger paint shall not have any of the health hazard classifications set out in 4.1 (hazard class 3.1 to 3.10).

NOTE Annex B provides a list of commonly used colorants that have been found to meet the requirements of 4.2, providing they meet the purity requirements specified. These fall into one or more of the following categories: Colourants permitted for use in food and/or cosmetics and other pigments which meet the general requirements of 4.1.

4.2.2 Finger paints shall not contain azo colourants that by cleavage of one or more azo groups can produce the primary aromatic amines listed in Tables 1 and 2 when tested in accordance with the test method specified in Annex C.

4.3 Preservatives

See A.3.

Finger paints shall be preserved using only the preservatives listed in Annex D. The maximum concentrations of preservatives shall not exceed the limits in column 4 of Table D.1 in Annex D.

Methods for the determination of certain preservatives are set out in the EU Cosmetics Legislation, (see Bibliography) and should be used when evaluating the maximum concentrations specified in the column “maximum allowed concentration” of Table D.1. Alternative methods that provide an equivalent detection limit may also be used.

4.4 Migration of certain elements

See A.4.

The concentration of elements shall not exceed the maximum limit values for finger paint specified in ISO 8124-3:2010, Table 1.

4.5 Limits for impurities

See A.5.

4.5.1 Limits for primary aromatic amines

4.5.1.1 The primary aromatic amines listed in Table 1 shall not be detectable when tested in accordance with the test method in Annex C.

Table 1 — Primary aromatic amines

Primary aromatic amines	**CAS Number**
Benzidine	92–87–5
2-Naphthylamine	91–59–8
4-Chloro-2-methylaniline (4-Chloro-*o*-toluidine)	95–69–2
4-Aminobiphenyl	92–67–1

4.5.1.2 Finger paint shall not contain carcinogenic primary aromatic amines (see Table 2 for a non-exhaustive list of relevant substances) in a total amount exceeding 20 mg/kg, with no individual primary aromatic amine exceeding 10 mg/kg, when tested in accordance with Annex C. The limitation does not apply to aromatic aminocarboxylic acids or aminosulfonic acids.

Table 2 — Other primary aromatic amines (non-exhaustive list)

Primary aromatic amine	**CAS Number**
o-Aminoazotoluene (4-*o*-Tolyazo-*o*-toluidine)	97–56–3

[a] For certain pigments limit values for 3,3’-Dichlorobenzidine are given Annex B, Table B.1.

Table 2 *(continued)*

Primary aromatic amine	CAS Number
2-Amino-4-nitrotoluene (5-Nitro-*o*-toluidine)	99–55–8
4-Chloroaniline	106–47–8
2,4-Diaminoanisole	615–05–4
4,4'-Diaminodiphenylmethane (4,4'-Methylenedianiline)	101–77–9
3,3'-Dichlorobenzidine[a]	91–94–1
3,3'-Dimethoxybenzidine	119–90–4
3,3'-Dimethylbenzidine	119–93–7
3,3'-Dimethyl-4,4'-diaminodiphenylmethane (4,4'-Methylenedi-o-toluidine)	838–88–0
p-Cresidine (6-Methoxy-m-toluidine)	120–71–8
2,2'-Dichloro-4,4'-methylenedianiline (4,4'-Methylene-bis-2-chloroaniline)	101–14–4
4,4'-Oxydianiline	101–80–4
4,4'-Thiodianiline	139–65–1
o-Toluidine	95–53–4
2,4-Xylidine	95–68–1
2,6-Xylidine	87–62–7
4-Amino-3-fluorophenol	399–95–1
6-Amino-2-ethoxynaphthalene	not available
2-Methoxyaniline (*o*-Anisidine)	90–04–0
4-Aminoazobenzene	60–09–3
4-Methyl-*m*-phenylenediamine (Toluene-2,4-diamine)	95–80–7
2,4,5-Trimethylaniline	137–17–7
Aniline	62–53–3

[a] For certain pigments limit values for 3,3'-Dichlorobenzidine are given Annex B, Table B.1.

4.5.2 Limits for other impurities

See A.5.

Finger paints shall not contain the impurities specified in Table 3, column 1 above the limits specified in column 2 when colourants specified in column 3 are used, when tested in accordance with Annex E.

Table 3 — Impurities in finger paints

Values in milligrams per kilogram of finger paint

Impurity	Limit	Finger paints of concern
Polychlorinated biphenyls	< 2	Finger paints containing chlorinated colourants or colourants manufactured in chlorinated solvents
Hexachlorobenzene (CAS No. 118–74–1)	< 5	Finger paints containing chlorinated colourants or colourants manufactured in chlorinated solvents
Benzo (α) pyrene (CAS No. 50–32–8)	< 0,05	Only for finger paints containing carbon black

4.6 Taste and smell

See A.7.

Finger paints shall not be sweetened, flavoured or fragranced.

An embittering agent in accordance with Table 4 shall be added in order to discourage and minimize the ingestion of paint.

Table 4 — Embittering agents

Embittering agent	CAS Number
Naringin	CAS 10236–47–2
Denatonium benzoate	CAS 3734–33–6

The bitterness should be detectable by taste when the finger paint is diluted with water in a ratio of 1:100. The following levels have been found suitable: naringin 1 %; denatonium benzoate 0,0004 % (4 mg/kg). The relative bitterness of these substances is approximately 1:3 000 (naringin : denatonium benzoate).

The embittering agent and its concentration should be such that it provides a bitter taste during the expected lifetime of the finger paint.

4.7 pH value

See A.8.

The pH value of the finger paint shall be between 4,0 and 10,0 when tested in accordance with ISO 787-9.

4.8 Binding agents, extenders, humectants and surfactants

Finger paints shall only use binding agents, extenders, humectants and surfactants which are not classified with any of the health hazards set out in in 4.1 (hazards class 3.1 to 3.10).

NOTE See Annex F for a list of compounds known to be commonly used in finger paints.

4.9 N-Nitrosamines

See A.9.

Finger paints shall not contain more than 0,02 mg/kg of N-nitrosodiethanolamine (NDELA), CAS No.:1116-54-7.

NOTE One suitable and validated test method is detailed in EN 71–12:2013, 7.2.1 and Clause 8. Other test methods may be used provided they are validated for finger paints.

4.10 Container

See A.10.

Containers used for finger paints shall not have a design that is likely to be mistaken by children with containers of foodstuffs or drinks.

Manufacturers should take into consideration the material(s), shape, volume, colours, labelling, other packaging and the means of access to the contents, when assessing similarity with containers of foodstuff or drinks.

Annex A
(informative)

Rationale

A.1 Finger paints

In addition to water, *finger paints* essentially consist of colourants, binders, preservatives and embittering agents and may additionally contain extenders, humectants and surfactants. *Finger paints* may be coloured by using colouring substances or mixtures containing colouring and other ingredients which are incorporated into a finger paint to impart colour to the finger paint.

A.2 Colourants

See 4.2.

Annex B contains a list of colourants which are suitable for finger paints subject to them meeting certain purity requirements. It includes colourants specific to finger paints, food colourants and cosmetic colourants.

More detailed requirements on aromatic amines are included in order to exclude risks which may derive from impurities in colourants.

Dyes are intensely coloured or fluorescent organic substances only, which impart colour to a substrate by selective absorption of light. They are soluble and/or go through an application process which, at least temporarily, destroys any crystal structure by absorption, solution, and mechanical retention, or by ionic or covalent chemical bonds.

Pigments are coloured, black, white or fluorescent particulate organic or inorganic solids which usually are insoluble in, and essentially physically and chemically unaffected by, the vehicle or substrate in which they are incorporated. They alter appearance by selective absorption and/or by scattering of light. Pigments are usually dispersed in vehicles or substrates for application, as for instance in the manufacture or inks, paints, plastics or other polymeric materials. Pigments retain a crystal or particulate structure throughout the coloration process.

A.3 Preservatives

See 4.3.

The use of appropriate preservatives in water-based systems like finger paints is necessary in order to provide protection against the growth of bacteria, fungi and yeasts and maintain a hygienic compound.

Preservatives allowed for use in finger paints are listed in Annex D. The following properties were taken into consideration when drafting this list: high efficacy with respect to micro-organisms accompanied by suitable toxicological performance (e.g. low human toxicity, low volatility, low odour), suitable technical performance (e.g. solubility in water-based systems, stability against other ingredients, stability against chemical or physical influences) and suitable environmental performance [e.g. low adsorbable organic halogens (AOX), low persistence]. The list includes, for example, preservatives that are allowed for cosmetic use or for food use.

A.4 Elements

See 4.4.

The limit values and test methods for the migration of certain elements in finger paints are specified in ISO 8124-3, Table 1, row 3.

A.5 Limits for primary aromatic amines

See 4.5.1.1 and 4.5.1.2.

Table 1 lists the four primary aromatic amines which shall not be determinable in finger paints at the limit of quantification (LOQ) using the method described in Annex C using GC-MS as the detection technique. Table 2 lists 24 primary aromatic amines which were taken from Table 2 of EN 71-7:2014 entitled “Other carcinogenic primary aromatic amines (non-exhaustive list)”. Therefore, the listing of primary aromatic amines in Table 2 of this part of ISO 8124 has been described as a non-exhaustive list and does not preclude other primary aromatic amines from being analysed. The information provided in Chromatography (C.6.5) and Precision (C.8) includes the four primary aromatic amines listed in Table 1, and 18 primary aromatic amines from Table 2. The inclusion of 18 rather than all 23 primary aromatic amines in Table 2 reflects the difficulty in obtaining the suitable commercial standards at the time of writing this part of ISO 8124. The information in C.5 and C.8 should provide the competent analyst with indicative information in the determination of other primary aromatic amines where/when calibration standards are available. The limitation for primary aromatic amines does not apply to aromatic aminocarboxylic acids or aminosulfonic acids because they are considered to be harmless.

A.6 Limit for benzo(α)pyrene

See 4.5.2.

Benzo(α)pyrene in finger paints is limited to the currently technically achievable analytical limit of quantitation (LOQ) of 0,02 mg/kg.

A.7 Taste and smell

See 4.6.

The addition of sweeteners, flavourings and fragrances to a finger paint may be determined by an assessment of the individual ingredients in the formulation of the finger paint.

The requirement to add an embittering agent to finger paints is intended to minimize potential oral ingestion by young children who may deliberately or accidentally put some of the finger paint in their mouth.

It should be noted that products with added embittering agents show remarkable bitterness, which may remain for a while after putting it into the mouth.

Experience has shown that the bitterness in finger paint will last for the product’s lifetime, if one of the two embittering agents in Table 4 is used in diluted finger paint as given in the Note in 4.6.

A.8 pH value

See 4.7.

The use of calcium carbonate may raise the pH value as a result of more dissociation by dilution with water in accordance with ISO 787-9 up to pH 10.

Some types of formulations containing calcium carbonate show pH values higher than 10 because the dilution of the finger paint is made with distilled water. This may also be found for small amounts of calcium carbonate in finger paint. Such a high increase as an analytical artefact does not happen when tap water is used to dilute the finger paint.

A.9 N-Nitrosamines

See 4.9.

To avoid the possible formation of N-nitrosamines avoid using formulations that combine diethanolamine andtriethanolamine (which is known to be often contaminated with diethanolamine or which can be decomposedto diethanolamine) with possible sources of nitrite that may be present in certain preservatives (e.g. bronopol) and other raw materials.

Work undertaken during the development of EN 71-12:2013, showed that the only N-nitrosamine of relevance for finger paints is N-nitrosodiethanolamine (NDELA), CAS No.:1116-54-7. Consequently this part of ISO 8124 sets a limitation on NDELA only. EN 71-12:2013 mentions other N-nitrosamines because this aligns with the EU toy safety directive but other N-nitrosamines are not known to occur in finger paints.

EN 71-12:2013 contains a validated test method for the determinations of NDELA in finger paints but other test methods may be used provided they are validated for determination of NDELA in a typical finger paint matrix.

A.10 Containers

See 4.10.

This requirement is intended to minimize the potential for a child to mistake finger paint for a foodstuff or a drink. The requirement is one of a series of precautionary measures (e.g. embittering agents, no flavour, no sweetening, no fragrance, warning to parents to supervise) intended to discourage the ingestion of finger paints by typical users (e.g. two years and above).

A.11 Labelling guidelines

See Annex G.

Young children have a propensity for exploring objects orally. The warning phrase recognizes the fact that children under three years of age may be tempted to taste or eat the finger paint and this is something that the supervisor would be expected to discourage or prevent. Even though finger paints that are in conformity with this part of ISO 8124 may be considered to present a minimal risk, ingestion of repeated amounts of finger paint is not recommended.

Annex B
(informative)

Non exhaustive list of colourants that are commonly used in finger paints and need to be in compliance with both the general and specific purity requirements

Table B.1 lists organic colourants commonly used in finger paints.

Table B.1 — Organic colourants which are commonly used in finger paints

No.	Colour	CI Generic Name[a]	CI Constitution Number	CAS Number	Limitations, requirements and information
1	Yellow	Pigment Yellow 1	11680	2512–29–0	See [b]. Check purity criteria for amine limits and that excess coupling component[d] is less than 1 000 ppm (parts per million).
2		Pigment Yellow 3	11710	6486–23–3	See [b]. Check purity criteria for amine limits and that excess coupling component[d] is less than 1 000 ppm.
3		Pigment Yellow 12	21090	6358–85–6	3,3'-Dichlorobenzidine ≤ 5 mg/kg: see C.1 detection limit of the method.
4		Pigment Yellow 13	21100	5102–83–0	3,3'-Dichlorobenzidine ≤ 5 mg/kg: see C.1 detection limit of the method.
5		Pigment Yellow 14	21095	5468–75–7	3,3'-Dichlorobenzidine ≤ 5 mg/kg: see C.1 detection limit of the method.
6		Pigment Yellow 17	21105	4531–49–1	3,3'-Dichlorobenzidine ≤ 5 mg/kg: see C.1 detection limit of the method.
7		Pigment Yellow 74	11741	6358–31–2	Check purity criteria for amine limits and that excess coupling component[d] is less than 1 000 ppm.
8		Pigment Yellow 138	56300	30125–47–4	
9		Pigment Yellow 139	56298	36888–99–0	
10		Pigment Yellow 151	13980	31837–42–0	
11	Yellow	Pigment Yellow 154	11781	68134–22–5	Check purity criteria for amine limits and that excess coupling component[d] is less than 1 000 ppm.
12		Pigment Yellow 155	200310	68516–73–4	
13		Pigment Yellow 185	56290	76199–85–4	
14		Natural Yellow 3	75300	458–37–7	Food grade, for example see current European legislation (2008/128/EC), E 100
15		Natural Yellow 6	75100	89382–88–7 27876–94–4	

[a] The Colour Index is published by The Society of Dyers and Colourists, PO Box 244, Perkin House 82 Grattan Road, Bradford, West Yorkshire BD1 2JB, United Kingdom, www.colour-index.org. Colourants are classified using both their CI generic name and their constitution number. These relate only to the "essential colourant" as defined by the Colour Index.

[b] This substance is restricted in Regulation (EC) No 1223/2009[8] as follows: "Not to be used in eye products" or "Not to be used in products applied on mucous membranes", as applicable.

[c] This substance is restricted in Regulation (EC) No 1223/2009[8] as follows: "Rinse-off products".

d Azo colourants are typically formed by a reaction sequence of diazotization of a primary aromatic amine which is referred to as the diazo component, followed by reaction (known as "coupling") with a compound having active methylene groups referred to as a coupling component.

Table B.1 *(continued)*

No.	Colour	CI Generic Name[a]	CI Constitution Number	CAS Number	Limitations, requirements and information
16		Natural Yellow 26	75130	7235–40–8	Food grade, for example see current European Directive (2008/128/EC), E 160 a
17		Natural Yellow 27	75135	79–75–4 3763–55–1	Food grade, for example see current European Directive (2008/128/EC), E 161 d
18	Orange	Pigment Orange 13	21110	3520–72–7	Check purity criteria for amine limits and coupling component[d] limits Combinations with Pigment Black 7 shall be checked for release of 3,3’dichlorobenzene 3,3’-dichlorobenzidine ≤ 5 mg/kg: see C.1 Detection limit of the method
19	Orange	Pigment Orange 34	21115	15793–73–4	Check purity criteria for amine limits and coupling component[d] limits Combinations with Pigment Black 7 shall be checked for release of 3,3’ dichlorobenzene 3,3’-dichlorobenzidine ≤ 5 mg/kg: see C.1 Detection limit of the method
20		Pigment Orange 43	71105	4424–06–0	See [b].
21		Pigment Orange 71	561200	86432–50–8	
22		Pigment Orange 73	56117	84632–59–7	
23		Natural Orange 4	75120	8015–67–6	Food grade, for example see current European Directive (2008/128/EC), E 160 b
24	Red	Pigment Red 48:2	15865:2	7023–61–2	
25		Pigment Red 48:3	15865:3	15782–05–5	
26		Pigment Red 57:2	15850:2	17852–98–1	
27		Pigment Red 57:1	15850:1	5281–04–9	
28		Pigment Red 63:1	15880:1	6417–83–0	
29		Pigment Red 68	15525	5850–80–6	
30		Pigment Red 83	58000:1	104074–25–1	
31		Pigment Red 122	73915	980–26–7	See [c].
32		Pigment Red 181	73360	2379–74–0	
33		Pigment Red 214	200660	82643–43–4	
34		Pigment Red 242	20067	52238–92–3	
35		Pigment Red 254	56110	84632–65–5	
36		Pigment Red 255	561050	54660–00–3	
37		Pigment Red 264	561300	88949–33–1	
38		Pigment Red 272	561150	350249–32–0	

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[b] This substance is restricted in Regulation (EC) No 1223/2009[8] as follows: “Not to be used in eye products” or “Not to be used in products applied on mucous membranes”, as applicable.

[c] This substance is restricted in Regulation (EC) No 1223/2009[8] as follows: “Rinse-off products”.

[d] Azo colourants are typically formed by a reaction sequence of diazotization of a primary aromatic amine which is referred to as the diazo component, followed by reaction (known as “coupling”) with a compound having active methylene groups referred to as a coupling component.

Table B.1 *(continued)*

No.	Colour	CI Generic Name[a]	CI Constitution Number	CAS Number	Limitations, requirements and information
39	Red	Natural Red 4	75470	1390–65–4 1260–17–9	Food grade, for example see current European Directive (2008/128/EC), E 120
40	Violet	Pigment Violet 19	73900	1047–16–1	See [c].
41		Pigment Violet 23	51319	215247–95–3 6358–30–1	See [c].
42	Blue	Pigment Blue 15	74160	147–14–8, 12239–87–1 27614–71–7 68987–63–3 16040–69–0	Check purity criteria for PCB, PCDD/DF and HCB limits. For example, limits as specified in existing chemical regulations in EU (see regulation. (EC) No. 1907/2006 "REACH", Annex XVII) Pigment Blue 15:2 and 15:4 are surface treated. Before using surface treated substances, manufacturers should contact their suppliers regarding composition and hazards.
43		Pigment Blue 16	74100	574–93–6	See [c].
44		Pigment Blue 60	69800	81–77–6	
45	Green	Pigment Green 7	74260	1328–53–6	See [b].
46		Pigment Green 36	74265	14302–13–7	
47		Natural Green 3	75810	8049–84–1 11006–34–1	Food grade, for example see current European Directive (2008/128/EC), E 140 and E 141

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[c] This substance is restricted in Regulation (EC) No 1223/2009[8] as follows: "Rinse-off products".

[d] Azo colourants are typically formed by a reaction sequence of diazotization of a primary aromatic amine which is referred to as the diazo component, followed by reaction (known as "coupling") with a compound having active methylene groups referred to as a coupling component.

Table B.2 lists non-organic colourants commonly used in finger paints.

Table B.2 — Non-organic colourants which are commonly used in finger paints

No.	Colour	CI Generic Name[a]	CI Constitution Number	CAS Number	Limitations, requirements and information
48	Yellow	Pigment Yellow 42	77492	51274–00–1	Iron oxide yellow Food grade, for example see current European Directive (2008/128/EC), E 172
49	Red	Pigment Red 101	77491	1309–37–1	Food grade, for example see current European Directive (2008/128/EC), E 172
50	Blue	Pigment Blue 29	77007	1317–97–1 57455–37–5	Ultramarine

[a] The Colour Index is published by The Society of Dyers and Colourists, PO Box 244, Perkin House 82 Grattan Road, Bradford, West Yorkshire BD1 2JB, United Kingdom, www.colour-index.org. Colourant are classified using both their CI Generic name and their constitution number. These relate only to the "essential colourant" as defined by the Colour Index.

Table B.2 *(continued)*

No.	Colour	CI Generic Name[a]	CI Constitution Number	CAS Number	Limitations, requirements and information
51	White	Pigment White 4	77947	1314–13–2	Zinc Oxide
52		Pigment White 6	77891	13463–67–7	Food grade, for example see current European Directive (2008/128/EC), E 171
53		Pigment White 18	77220	207–439–9 208–915–9	Chalk Food grade, for example see current European Directive (2008/128/EC), E 170 Mixture Calcium carbonate, Magnesium carbonate
54		Pigment White 19	77004 77005	8047–76–5	Aluminium silicate, hydrated
55		Silver	77820	7440–22–4	Food grade, for example see current European Directive (2008/128/EC), E 174
56		Pigment White 21	77120	7727–43–7	Blancfixe
57		Pigment White 25	77231	91315–45–6	Gypsum
58		Aluminium, zinc, magnesium and calcium stearates	-	Various	
59	Black	Pigment Black 6	77266	1333–86–4	Carbon black food contact quality
60		Pigment Black 7	77266	1333–86–4	Carbon black food contact quality
61		Pigment Black 11	77499	12227–89–3	Food grade, for example see current European Directive (2008/128/EC), E 172
62	Brown	Pigment Metal 3	77480	7440–57–5	Gold Food grade, for example see current European Directive (2008/128/EC), E 175
63	Orange	Ferrous oxide	77489	Various	Food grade, for example see current European Directive (2008/128/EC), E 172 (Mix)

[a] The Colour Index is published by The Society of Dyers and Colourists, PO Box 244, Perkin House 82 Grattan Road, Bradford, West Yorkshire BD1 2JB, United Kingdom, www.colour-index.org. Colourant are classified using both their CI Generic name and their constitution number. These relate only to the “essential colourant” as defined by the Colour Index.

Annex C
(normative)

Method for the detection of certain azo colourants and determination of free primary aromatic amines

C.1 General

For the detection of certain azo *colourants*, the sample is treated with sodium dithionite in a citrate buffer (pH 6) at 70 °C in a sealed vessel. Upon reductive cleavage, the resultant amines are extracted with *tert*-Butyl methyl ether (MTBE) by means of a “kieselguhr” type SPE column. The ether extract is carefully concentrated with a rotary evaporator or an equivalent sample concentrator and the residue is dissolved in acetonitrile or other suitable solvent, depending upon the detection/determination procedure to be used.

The detection/determination of amines resulting either from the reductive cleavage method or already present in the sample as free primary aromatic amines is performed by high performance liquid chromatography with a diode-array detector (HPLC/DAD) or by capillary gas chromatography with mass-selective detector (GC-MS).

The amines shall be identified by at least one of the chromatographic separation techniques described in this annex. Unless an unequivocal identification is achieved (e.g. by using GC-MS and comparing retention times with known standards), confirmation of positive results shall be achieved by a suitable alternative separation technique (to avoid possible misinterpretation from, for example, isomers of the amines to be identified).

NOTE 1 Some amines may be thermally unstable and cannot be determined by gas chromatography.

The quantification of the amines is performed by HPLC/DAD or GC-MS.

NOTE 2 Some of the amines are cleaved under the reductive conditions in C.6.2 according to Table C.1:

Table C.1 — Amine compounds and cleavage products

Amine compound	Cleavage products
o-Aminoazotoluene	*o*-Toluidine, 2-Methyl-*p*-phenylendiamine
2-Amino-4-nitrotoluene	4-Methyl-*m*-phenylenediamine
4-Aminoazobenzene	*p*-Phenylenediamine, Aniline

4-Aminoazobenzene is reductively cleaved to *p*-phenylenediamine and aniline; *o*-aminoazotoluene is reductively cleaved to 2-methyl-*p*-phenylenediamine and *o*-toluidine; and 5-nitro-*o*-toluidine is reduced to 4-methyl-*m*-phenylenediamine.

A prohibited azo colourant is deemed to be present in the finger paint if, on reductive cleavage, one or more of the amines listed in Tables 1 and 2 is present in a concentration exceeding 30 mg/kg.

Each primary aromatic amines listed in Table 1 is deemed to be determinable at levels exceeding 5 mg/kg.

C.2 Reagents

Reagent-grade chemicals are to be used, if nothing else is specified.

C.2.1 Methanol.

C.2.2 Acetonitrile.

C.2.3 *tert*-Butyl methyl ether.

C.2.4 Citrate/sodium hydroxide buffer, *c*(trisodium citrate) = 0,06 mol/l, pH 6, preheated to 70 °C or 37 °C: Dissolve 12,6 g citric acid monohydrate and 6,4 g sodium hydroxide in 900 ml water. Adjust the volume to 1 L1).

C.2.5 Sodium dithionite solution, freshly dissolved in water, ρ = 200 mg/ml.

C.2.6 Porous, granular "kieselguhr" SPE column2).

C.2.7 Anhydrous sodium sulfate.

C.2.8 Certified amine standards, primarily those listed in Tables 1 and 2.

NOTE The amines in Tables 1 and 2 are human carcinogens or cancer suspect agents (Cat. 1A and 1B). The handling of these chemicals requires the utmost care and commensurate safety measures.

C.2.9 Internal standards for gas chromatography.

C.2.9.1 IS 1: 2,4,5-Trichloroaniline, CAS No. 636-30-6.

C.2.9.2 IS 2: 4-Amino-2-methylquinoline, CAS No. 6628-04-2.

C.2.9.3 IS 3: Tributylphosphate, CAS No. 126-73-8.

C.2.10 Standard solutions.

C.2.10.1 Stock solution of aromatic amines.

Prepare a stock solution containing 100 mg/l of each aromatic amine (C.2.8) in methanol (C.2.1). This solution shall be stored in the absence of light at (−18 ± 2) °C.

C.2.10.2 Calibration solutions.

Prepare six calibration solutions in the range 0,1 mg/l to 5,0 mg/l by dilution of the aromatic amines stock solutions (C.2.10.1) into (100 ± 0,1) ml volumetric flasks using MBTE (C.2.3). Before making up to the final volume, add 0,1 ml of internal standard (C.2.10.3) to each calibration solution in order to obtain a final internal standard concentration of 1 mg/l. The calibration solutions are ready for GC-MS analysis.

C.2.10.3 Internal standard solution.

Prepare a stock solution of each internal standard (C.2.9.1 – C.2.9.3) at 10,0 mg/l in methanol (C.2.1).

C.2.10.4 Recovery solution of aromatic amines.

Prepare a recovery solution containing 10 mg/l of each aromatic amine (C.2.10.1) in methanol (C.2.1). This solution shall be stored in the absence of light at (−18 ± 2) °C.

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1) "Ready for use" solution, Merck-Nr. 1.09437 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.

2) Chromabond® XTR 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.

The stability of all calibration solutions should be checked regularly. These should be stable for up to 6 months when stored in the dark at (−18 ± 2) °C.

NOTE The solvent used will depend on the chromatographic method chosen for analysis.

C.3 Apparatus

Ordinary laboratory equipment, and:

C.3.1 Reaction vessel (50 ml conical flask) of temperature-resistant glass with tight-fitting cap.

C.3.2 Water bath, capable of maintaining a temperature of (37 ± 2) °C and (70 ± 2) °C.

C.3.3 Column made from glass or polypropylene, 25 mm to 30 mm internal diameter, 140 mm to 150 mm length, filled with about 20 g porous, granular “kieselguhr” SPE material, fitted on the outlet with a glass fibre filter (or commercial SPE column)[3].

C.3.4 Vacuum rotary evaporator or equivalent low temperature sample concentration system.

C.3.5 Pipettes 10 ml, 5 ml, 2 ml, 1 ml.

C.4 Instrumentation

The analysis shall be performed using equipment selected from the following list.

C.4.1 HPLC with gradient-elution and DAD.

C.4.2 GC with MS.

C.5 Sampling procedure

Homogenize the sample by stirring thoroughly.

C.6 Procedure

C.6.1 Sample preparation

For both the detection of certain azo colourants, and the determination of “free” primary aromatic amines, a representative sample of approximately 1,0 g is weighed accurately into a 50 ml conical flask (C.3.1).

C.6.2 Reductive cleavage of azo colourants

Approximately 15 ml of buffer (C.2.4), preheated to (70 ± 2) °C, is added to the sample. The conical flask is tightly closed and after brief vigorous shaking to homogenize the contents is kept at (70 ± 2) °C for (30 ± 2) min.

To achieve reductive cleavage of the azo colourants (3,0 ± 0,01) ml of sodium dithionite solution (C.2.5) is added to the conical flask. The conical flask is immediately tightly sealed, thoroughly shaken and kept again at (70 ± 2) °C for another (30 ± 2) min, and then cooled to ambient temperature within 2 min.

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[3] Chromabond® XTR (Macherey-Nagel Catalogue No. 730507) 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.

C.6.3 Extraction of soluble amines

For the determination of “free” aromatic amines (see 4.5.1.1 and 4.5.1.2), the reductive cleavage (C.6.2) is not carried out. Instead, 20 ml of the buffer solution (C.2.4) preheated only to (37 ± 2) °C is added to the sample. The conical flask is tightly closed and after brief vigorous shaking to homogenize the contents, is kept at (37 ± 2) °C for about 30 min.

C.6.4 Solid phase extraction and concentration of amines

The solution from C.6.2 or C.6.3, as appropriate, is poured onto the SPE column without rinsing the conical flask with water or buffer. The aqueous phase is left for 30 min to absorb onto the column. The amines are then extracted twice with 40 ml *tert*-Butyl methyl ether as described below.

Before extracting the SPE column, the first 40 ml of *tert*-Butyl methyl ether is divided into portions of 2 × 10 ml and 1 × 20 ml for rinsing the conical flask. Then 10 ml of ether is added to the flask and it is closed and shaken vigorously. After allowing 30 min for the water phase to absorb onto the column, the *tert*-Butyl methyl ether is decanted from the conical flask onto the column leaving behind any residual water in the reaction vessel (Note: addition of 0,2 g of anhydrous sodium sulfate to dry the *tert*-Butyl methyl ether). The eluant is collected in a 100ml glass vessel for evaporation. This operation is repeated with the remaining 10 ml and 20 ml portions of *tert*-Butyl methyl ether. Finally, the second 40 ml is poured directly onto the column.

The *tert*-Butyl methyl extract is carefully concentrated at a maximum temperature of 25 °C using a rotary evaporator with vacuum, or equivalent sample concentrator, to about 1 ml (do not allow the solution to go to dryness). If *tert*-Butyl methyl is not the required chromatographic solvent, the remainder of the ether is carefully removed under a light flow of inert gas and the residue made up to 2,0 ml with acetonitrile in a graduated test tube. If *tert*-Butyl methyl is the required chromatographic solvent, the residue is quantitatively transferred to a small graduated tube and the volume made up to (2,0 ± 0,1) ml using washings from the container used in the rotary evaporator flask or sample concentrator.

During solvent removal, considerable losses of amines may occur if the process is not closely supervised (i.e. vacuum too high, temperature too high, high inert gas flow). The solvent removal should be performed under subdued light (avoid direct sunlight and if possible, direct fluorescent lightening).

If taken to dryness, each residue is immediately dissolved in (2,0 ± 0,1) ml of a suitable solvent, e.g. methanol in an amber glass flask, and subsequently analysed. If the analysis cannot be carried out immediately, the sample has to be stored at (−20 ± 2) °C.

The quantification of the amines is conducted using HPLC/DAD or GC-MS. If using GC-MS, internal standards shall be used.

NOTE Certain amines, e.g. 2,4-toluenediamine and 2,4-diaminoanisole, have a very low stability. If the extraction and concentration procedure is not carried out expediently, partial or total loss of amines can occur.

C.6.5 Chromatography

C.6.5.1 General

The following conditions have been found suitable for the detection/determination of primary aromatic amines. The analysis of finger paints shall be performed in accordance with the methods of analysis described in this part of ISO 8124. Alternative methods of analysis or modifications to the procedures described are acceptable only if they are capable of achieving at least the accuracy and precision of the methods described in this part of ISO 8124 i.e. have an adequate sensitivity and have been validated to show that the results are equivalent to those of these standard methods.

NOTE It is reported that some amines are heat sensitive and may breakdown on heating when injected onto a GC column, requiring an alternative detection technique to be used.

C.6.5.2 High pressure liquid chromatography (HPLC)

Eluent 1	Acetonitrile
Eluent 2	0,575 g ammonium dihydrogenphosphate + 0,7 g disodium hydrogenphosphate in 1 000 ml water, pH 6,9
Column	HyPurity Advance 250 mm x 3 mm; 5 µm ThermoQuest Catalogue No. 21005-0035
Flow rate	0,5 ml/min
Gradient	0 min 15 % eluent 1, within 45 min linear to 75 % eluent 1
Column temperature	40 °C
Injection volume	5,0 µl
Detection	DAD, full spectral scan
Quantification	at 240 nm, 280 nm and 305 nm (see Figures C.1 to C.3)

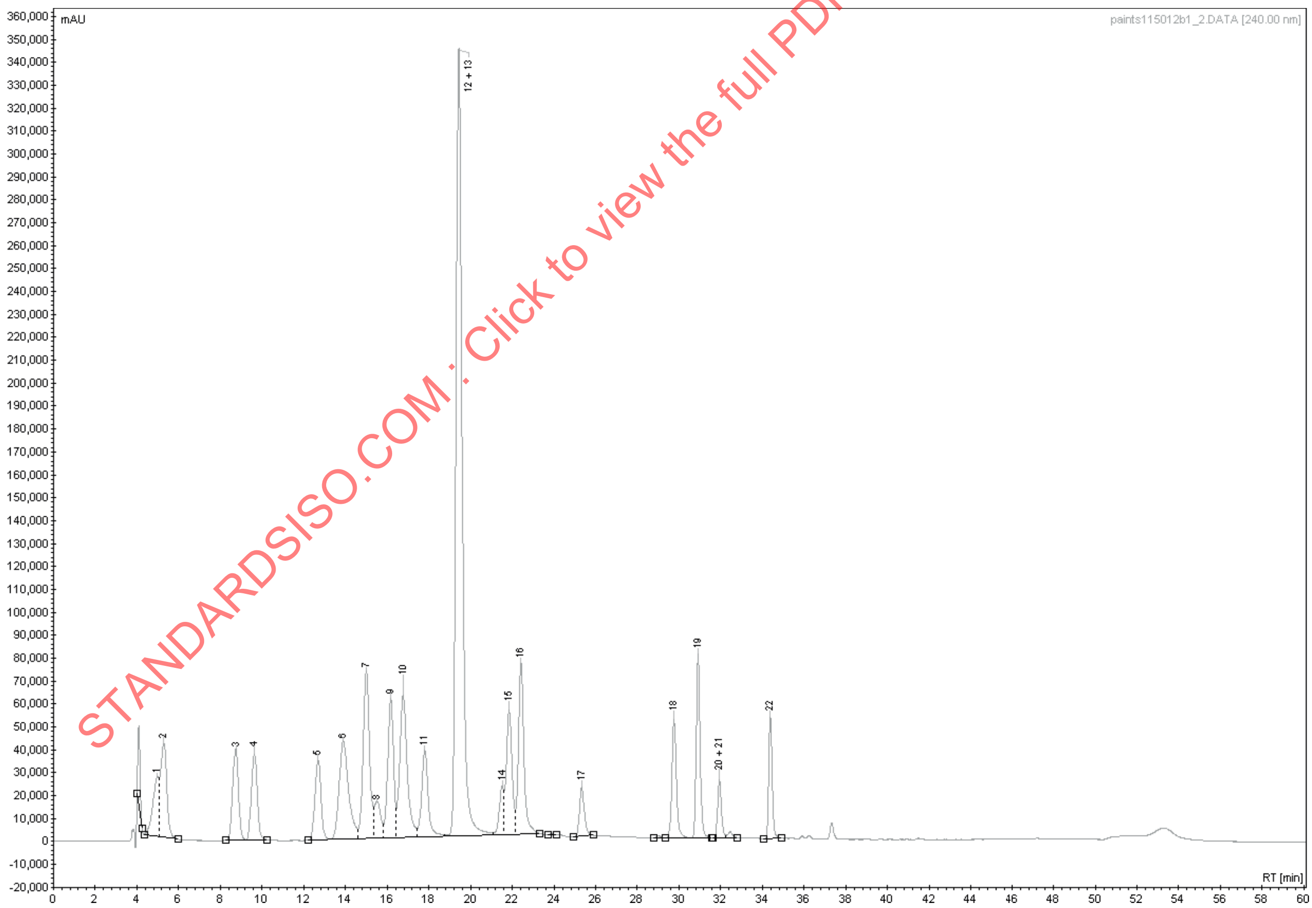

Figure C.1 — Example of HPLC-DAD chromatogram of 22 mixed aromatic amine standard at 240 nm using the conditions in C.6.5.1

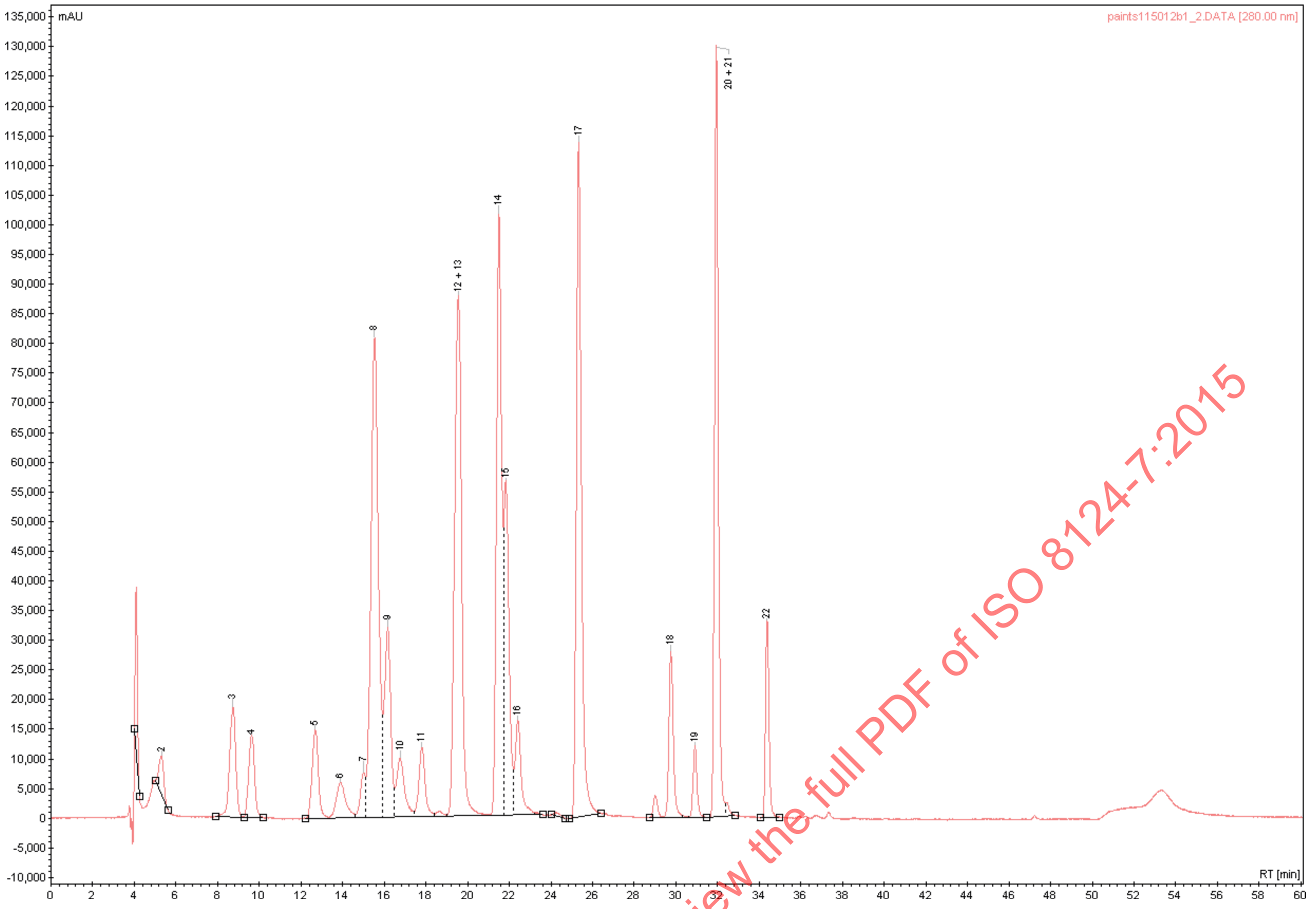

Figure C.2 — Example of HPLC-DAD chromatogram of 22 mixed aromatic amine standard at 280 nm using the conditions in C.6.5.1

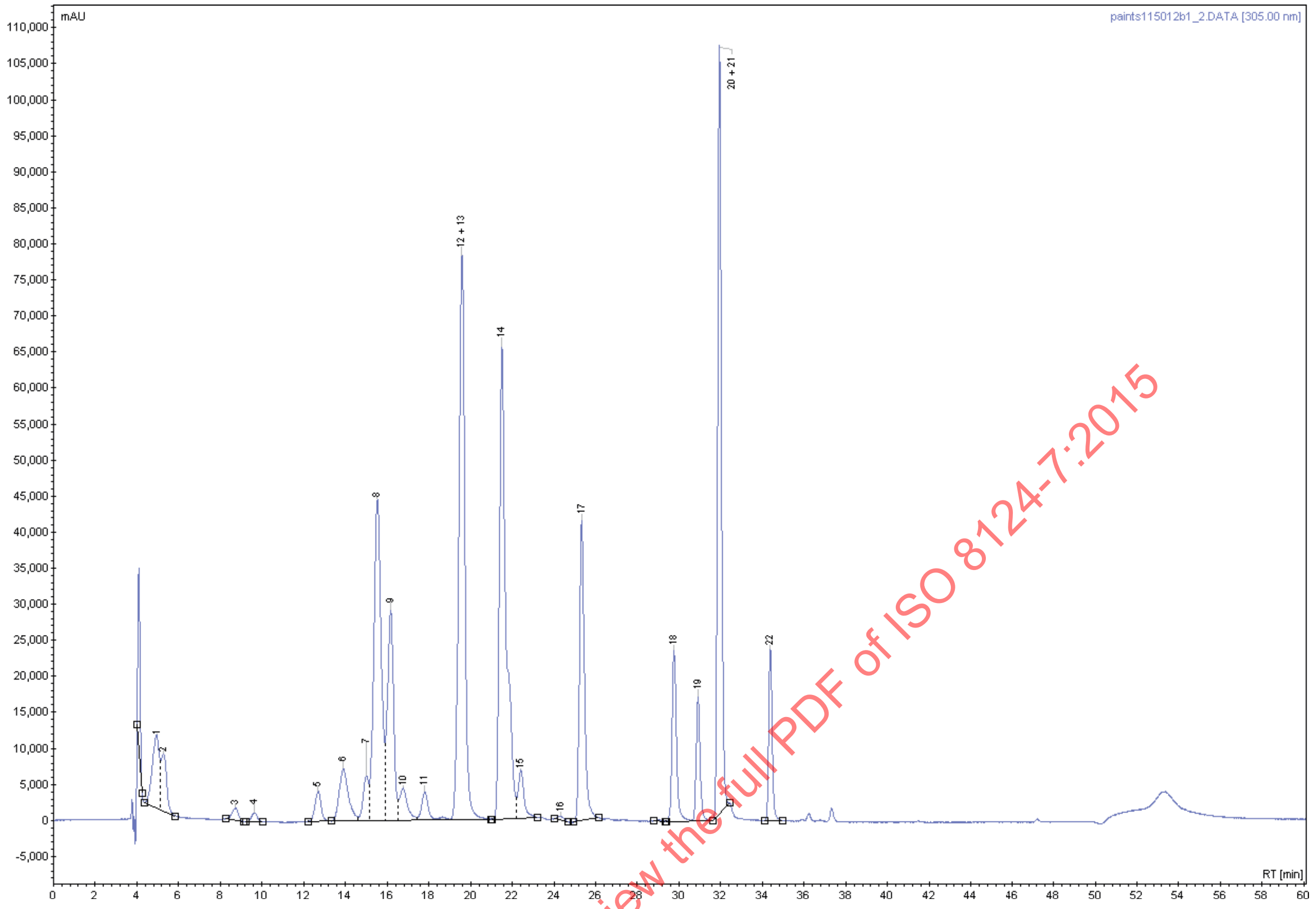

Figure C.3 — Example of HPLC-DAD chromatogram of 22 mixed aromatic amine standard at 305 nm using the conditions in C.6.5.1

Table C.2 — Key to retention time for LC-DAD at 280nm

No.	Retention time min	Primary aromatic amine	No.	Retention time min	Primary aromatic amine
1	4,97	4-Methoxy-*m*-phenylenediamine	12	19,4	4-Chloro-*o*-toluidine
2	5,29	4-Methyl-*m*-phenylenediamine	13	19,4	2-Naphthylamine
3	8,75	4-Methoxyaniline	14	21,52	3,3-Dimethoxybenzidine
4	9,64	*o*-Toluidine	15	21,85	3,3-Dimethylbenzidine
5	12,69	6-Methoxy-*m*-toluidine	16	22,43	4,4-Thiodianiline
6	13,89	4,4-Oxydianiline	17	25,33	4,4-Methylenedi-*o*-toluidine
7	14,99	Benzidine	18	29,01	4-Aminobiphenyl
8	15,50	4-Chloroaniline	19	30,80	*p*-Aminoazobenzene
9	16,10	5-Nitro-*o*-toluidine	20	32,45	2,2-Dichloro-4,4-methylenedianiline
10	16,7	4,4-Methylenedianiline	21	32,45	3,3-Dichlorobenzidine
11	17,8	2,4,5-Trimethylaniline	22	34,37	*o*-Aminoazotoluene

C.6.5.3 Gas Chromatography (GC) with mass spectrometry

Capillary column	RTX5 Amine or equivalent type, length: 30 m, internal diameter: 0,25 mm, film thickness: 0,25 µm, preferably deactivated for amines
Injector	split/splitless
Injection temperature	220 °C
Carrier	Helium
Temp. programme	60 °C (3 min), 60 °C to 280 °C (7 °C/min), 280 °C (4 min), 280°C to 300°C (10°C/min), 300°C (2 min)
Injection volumes	1,0 µl, split 1:15

A single quadrupole MS instrument is recommended with ionization by electronic impact at 70 eV.

GC interface temperature	250 °C
Source temperature	200 °C

In SIM mode, the fragment ions allow quantification by using one of the three ions as the target quantification ion and the remaining two ions as qualifiers identified in Table C.3.

NOTE T1 is considered as the target ion for quantification.

The time spent on each ion (dwell time) shall be the same for all ions within a given window. The m/z values are rounded. Exact values should be used as SIM parameters.

Table C.3 — List of ions for quantification

Primary aromatic amine	Target ion m/z	Qualifier 1 m/z	Qualifier 2 m/z
o-Toluidine	106	107	77
4-Chloroaniline	127	129	92
4-Methoxy aniline	108	123	80
6-Methoxy-m-toluidine	122	137	94
2,4,5-Trimethylaniline	135	120	134
4-Chloro-o-toluidine	106	141	140
4-Methyl-m-phenylendiamine	121	122	94
4-Methoxy-m-phenylenediamine	123	138	95
2-Naphthylamine	143	115	116
5-Nitro-o-toluidine	152	106	78
4-Aminobiphenyl	169	168	170
p-Aminoazobenzene	92	197	120
4,4’-Oxydianiline	200	108	171
Benzidine	184	183	185
4,4-Methylenedianiline	198	197	106
o-Aminoazotoluene	106	225	134
4,4-Methylenedi-o-toluidine	226	211	225
3,3-Dimethylbenzidine	212	213	106
4,4’-Thiodianiline	216	184	215

Table C.3 *(continued)*

Primary aromatic amine	Target ion m/z	Qualifier 1 m/z	Qualifier 2 m/z
3,3'-Dichlorobenzidine	252	254	126
2,2-Dichloro-4,4-methylenedianiline	231	266	195
3,3-Dimethoxybenzidine	244	201	229
2,4,5-Trichloroaniline	195		
4-Amino-2-methylquinoline	158		
Tributyl phosphate	99		

C.6.5.4 Maximum permitted tolerances

The relative intensities of the ion for quantification against respectively the two qualifiers, expressed as a percentage of the intensity of the most intense ion, shall correspond to those of the calibration standard solutions, at comparable concentrations, measured under the same conditions, within the tolerances described Table C.4.

The calibration standard used as reference should be at the middle of the calibration curve.

Table C.4 — Maximum permitted tolerances for relative ion intensities

Relative intensity % of base ion intensity	Relative range of the response
> 50 %	±10 %
> 20 % - 50 %	±15 %
> 10 % - 20 %	±20 %
≤ 10 %	±50 %

Figure C.4 shows an example of GC-MS chromatogram of 22 mixed aromatic amines. Table C.5 shows a key to retention time for GC-MS.

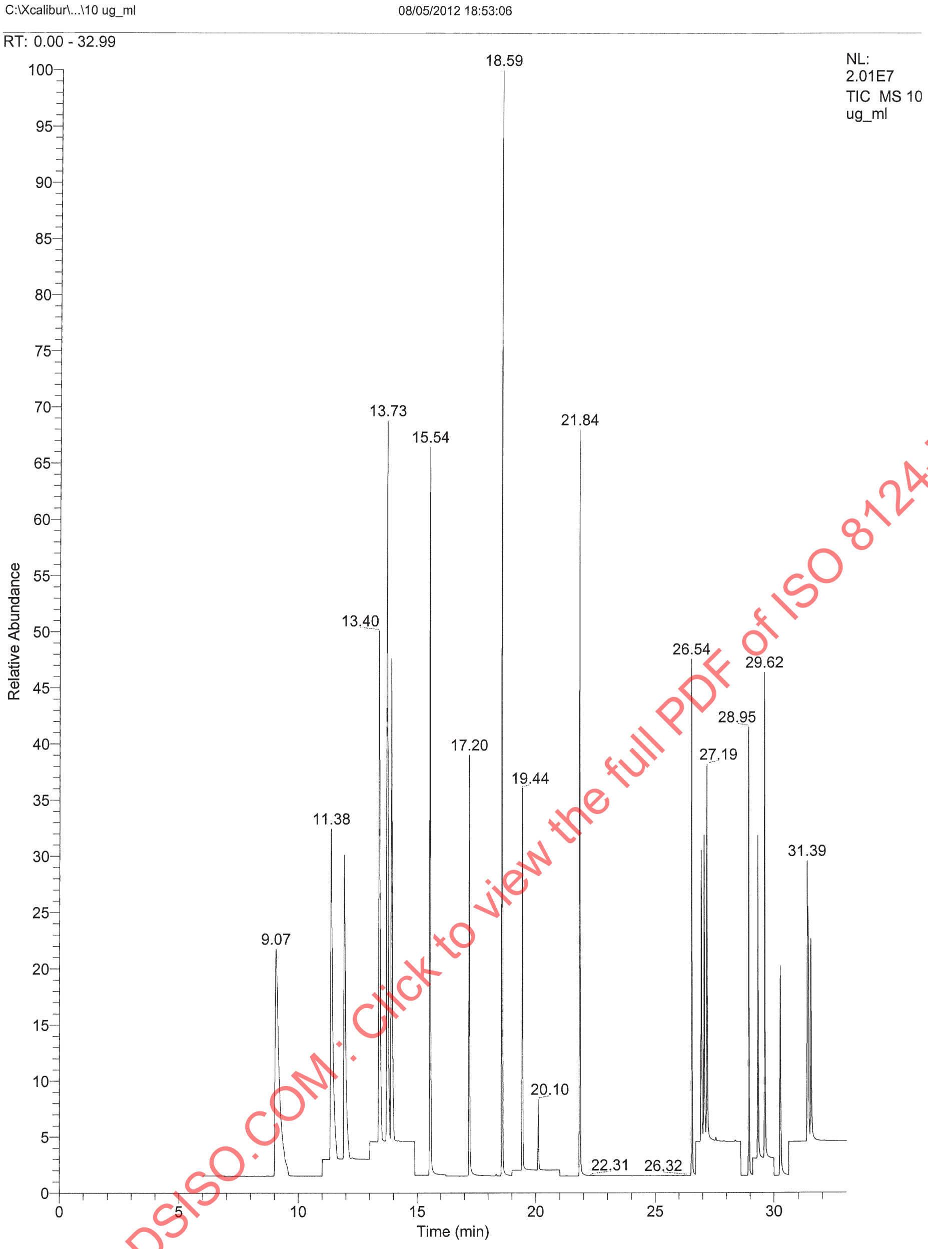

Figure C.4 — Example of GC-MS chromatogram of 22 mixed aromatic amines

Table C.5 — Key to retention time for GC-MS

No	Retention time (min)	Primary aromatic amine	No	Retention time (min)	Primary aromatic amine
1	9,07	*o*-Toluidine	12	26,54	*p*-Aminoazobenzene
2	11,38	4-Methoxyaniline	13	26,94	4,4-Oxydianiline
3	11,93	4-Chloroaniline	14	27,07	Benzidine
4	13,40	6-Methoxy-*m*-toluidine	15	27,19	4,4-Methylenedianiline
5	13,73	2,4,5-Trimethylaniline	16	28,95	*o*-Aminoazotoluene
6	13,92	4-Chloro-*o*-toluidine	17	29,33	4,4-Methylenedi-*o*-toluidine
7	15,53	4-Methyl-*m*-phenylenediamine	18	29,62	3,3-Dimethylbenzidine
8	17,20	4-Methoxy-*m*-phenylenediamine	19	30,26	4,4-Thiodianiline
9	18,58	2-Naphthylamine	20	31,39	3,3-Dichlorobenzidine
10	19,43	5-Nitro-*o*-toluidine	21	31,42	2,2-Dichloro-4,4-methylenedianiline
11	21,84	4-Aminobiphenyl	22	31,53	3,3-Dimethoxybenzidine

C.6.6 Verification of analytical system

To check the analytical procedure, add 0,2 ml of standard solution (C.2.10.4) and 1,0 ml methanol to reaction vessel C.3.1 containing 15 ml buffer preheated to (70 ± 2) °C. Then follow the procedure from C.6.2 (second sentence). The recovery rate of the amines will normally be expected to be ≥ 70 % with the exception of 4-methyl-m-phenylenediamine, 4-methoxy-m-phenylenediamine, 2-naphthylamine, p-aminoazobenzene and o- aminoazotoluene where the recoveries have been found to be between 10 % and 50 %.

C.7 Calculation

The amine concentration is calculated from the area of each amine peak and is given as mass portion *w*, in mg/kg, of single amine component in test material according to Formula (1):

$$w = \frac{A_s \cdot C_c \cdot V_s}{A_c \cdot E_s} \tag{C.1}$$

where

A_S is the peak area of the amine in the sample solution in area units;

A_C is the peak area of the amine in the calibration solution in area units;

C_C is the concentration of the amine in the calibration solution (µg/ml);

E_S is the initial mass of sample in the end volume (g);

V_S is the volume of test solution obtained in C.6.4 used for chromatographic analysis (ml).

If an internal standard has been used, the mass portion of amine component (*w*) is given as:

$$w = \frac{A_{IS}(S)}{A_{IS}(C)} \tag{C.2}$$

where

w is the mass portion of a single amine component in test material, in mg/kg;

$A_{is(s)}$ is the peak area of the internal standard in the sample solution in area units;

$A_{is(c)}$ is the peak area of the internal standard in the calibration solution in area units.

C.8 Precision

C.8.1 Linearity

The correlation coefficient shall be > 0,990.

C.8.2 Limits of detection (LOD) and quantification (LOQ)

The LODs and the LOQs for method using GC-MS and LC-DAD instruments are shown in Tables C.6 and C.7.

Table C.6 — Limits of detection (LOD) and quantification (LOQ) Using GC-MS

Primary aromatic amine	LOD mg/l	LOQ mg/kg	Primary aromatic amine	LOD mg/l	LOQ mg/kg
o-Toluidine	0,02	0,5	*p*-Aminoazobenzene	0,02	0,5
4-Methoxyaniline	0,02	0,5	4,4-Oxydianiline	0,05	1,0
4-Chloroaniline	0,02	0,5	Benzidine[a]	0,05	0,5
6-Methoxy-*m*-toluidine	0,02	0,5	4,4-Methylenedianiline	0,05	1,0
2,4,5-Trimethylaniline	0,02	0,2	*o*-Aminoazotoluene	0,02	0,5
4-Chloro-*o*-toluidine[a]	0,02	0,5	4,4-Methylenedi-*o*-toluidine	0,05	1,0
4-Methyl-*m*-phenylenediamine	0,02	0,2	3,3-Dimethylbenzidine	0,04	1,0
4-Methoxy-*m*-phenylenediamine	0,05	0,5	4,4-Thiodianiline	0,04	1,0
2-Naphthylamine[a]	0,01	0,2	3,3-Dichlorobenzidine	0,05	1,0
5-Nitro-*o*-toluidine	0,05	1,0	2,2-Dichloro-4,4-methylenedianiline	0,05	1,0
4-Aminobiphenyl[a]	0,01	0,2	3,3-Dimethoxybenzidine	0,05	1,0

[a] Primary aromatic amines that shall not be determinable when tested in accordance with the test method in Annex C.

Table C.7 — Limits of detection (LOD) and quantification (LOQ) using LC-DAD at 240 nm

Primary aromatic amine	LOD mg/l	LOQ mg/kg	Primary aromatic amine	LOD mg/l	LOQ mg/kg
o-Toluidine	1,0	2,0	*p*-Aminoazobenzene	1,0	2,0
4-Methoxyaniline	1,0	2,0	4,4-Oxydianiline	1,0	2,0
4-Chloroaniline	1,0	2,0	Benzidine	-	-
6-Methoxy-*m*-toluidine	1,0	2,0	4,4-Methylenedianiline	1,0	2,0
2,4,5-Trimethylaniline	1,0	2,0	*o*-Aminoazotoluene	1,0	2,0
4-Chloro-*o*-toluidine	-	-	4,4-Methylenedi-*o*-toluidine	1,0	2,0
4-Methyl-*m*-phenylenediamine	1,0	2,0	3,3-Dimethylbenzidine	1,0	2,0
4-Methoxy-*m*-phenylenediamine	1,0	2,0	4,4-Thiodianiline	1,0	2,0
2-Naphthylamine	-	-	3,3-Dichlorobenzidine	1,0	2,0
5-Nitro-*o*-toluidine	1,0	2,0	2,2-Dichloro-4,4-methylenedianiline	1,0	2,0
4-Aminobiphenyl	-	-	3,3-Dimethoxybenzidine	1,0	2,0

C.8.3 Repeatability (r) and reproducibility (R)

Each primary aromatic amine was determined in a single batch of finger paint containing 22 aromatic amines each spiked at a concentration of 1 mg/kg using C.6.3 by two independent laboratories on six replicate samples using GC-MS and shown in Table C.8.

Table C.8 — Repeatability and reproducibility data for soluble aromatic amines by GC-MS

Primary aromatic amine	Conc mg/kg	Lab 1 mg/kg	Lab 2 mg/kg
o-Toluidine	1,00	1,03 ± 0,17	0,81 ± 0,13
4-Methoxy-aniline	1,00	1,28 ± 0,16	0,68 ± 0,10
4-Chloroaniline	1,00	1,04 ± 0,13	0,63 ± 0,09
6-Methoxy-m-toluidine	1,00	1,17 ± 0.09	0,35 ± 0,07
2,4,5-Trimethylaniline	1,00	0.89 ± 0,14	0,44 ± 0,05
4-Chloro-o-toluidine	1,00	1,11 ± 0,18	0,87 ± 0,04
p-Aminoazobenzene	1,00	0,82 ± 0,10	0,16 ± 0,01
4,4'-Oxydianiline	1,00	0,83 ± 0,10	0,95 ± 0,07
Benzidine	1,00	0,72 ± 0,09	0,74 ± 0,06
4,4'-Methylenedianiline	1,00	0,72 ± 0,09	0,60 ± 0.06
o-Aminoazotoluene	1,00	0,99 ± 0,09	0,12 ± 0,01
4,4-Methylenedi-o-toluidine	1,00	1,02 ± 0,09	0,16 ± 0,01
4-Methyl-m-phenylenediamine	1,00	Not detected	0,15 ± 0,05
4-Methoxy-m-phenylenediamine	1,00	Not detected	0,11 ± 0,03
2-Naphthylamine	1,00	0,69 ± 0,03	0,18 ± 0,01
5-Nitro-o-toluidine	1,00	1,62 ± 0,03	0,69 ± 0,03
4-Aminobiphenyl	1,00	0,80 ± 0,14	0,47 ± 0,02
3,3'-Dimethylbenzidine	1,00	0,91 ± 0,17	0,24 ± 0,01
4,4'-Thiodianiline	1,00	0,94 ± 0,07	0,22 ± 0,03
3,3'-Dichlorobenzidine	1,00	1,28 ± 0,15	0,83 ± 0,06
2,2'-Dichloro-4,4-methylenedianiline	1,00	1,33 ± 0,12	0,77 ± 0.06
3,3'-Dimethoxybenzidine	1,00	1,36 ± 0,21	0,24 ± 0,01

C.8.4 Repeatability and reproducibility data for reductively cleaved aromatic amines

Each cleaved aromatic amine was determined in a single batch of finger paint containing 22 aromatic amines each spiked at 0,5 mg/kg using C.6.2 by two independent laboratories on six replicate samples using GC-MS and shown in Table C.9.

Table C.9 — Repeatability and reproducibility of cleaved aromatic amines by GC-MS

Reductively cleaved aromatic amine	Conc mg/kg	Lab 1 mg/kg	Lab 2 mg/kg
o-Toluidine	0,5	0,73 ± 0,09	0,33 ± 0,03
4-Methoxy-aniline	0,5	0,48 ± 0,02	0,32 ± 0.03
4-Chloroaniline	0,5	0,42 ± 0,03	0,30 ± 0.05
6-Methoxy-m-toluidine	0,5	0,51 ± 0,02	0,19 ± 0,02
2,4,5-Trimethylaniline	0,5	0,32 ± 0,04	0,24 ± 0,02

Table C.9 *(continued)*

Reductively cleaved aromatic amine	**Conc** mg/kg	**Lab 1** mg/kg	**Lab 2** mg/kg
4-Chloro-o-toluidine	0,5	0,40 ± 0,03	0,41 ± 0,04
4-Methyl-m-phenylenediamine	0,5	Not detected	0,15 ± 0,02
4-Methoxy-m-phenylenediamine	0,5	Not detected	0,19 ± 0,02
2-Naphthylamine	0,5	0,28 ± 0,03	0,17 ± 0,01
5-Nitro-o-toluidine	0,5	Not detected	0,28 ± 0,02
4-Aminobiphenyl	0,5	0,36 ± 0,03	0,22 ± 0,01
p-Aminoazobenzene	0,5	Not detected	0,17 ± 0,01
4,4’-Oxydianiline	0,5	0,72 ± 0.05	0,78 ± 0,10
Benzidine	0,5	0,55 ± 0.03	0,53 ± 0.07
4,4’-Methylenedianiline	0,5	Not detected	0,34 ± 0.06
o-Aminoazotoluene	0,5	Not detected	0,14 ± 0,01
4,4-Methylenedi-o-toluidine	0,5	0,74 ± 0,04	0,20 ± 0,01
3,3’-Dimethylbenzidine	0,5	0,55 ± 0.04	0,24 ± 0,02
4,4’-Thiodianiline	0,5	0,77 ± 0.05	0,23 ± 0,01
3,3’-Dichlorobenzidine	0,5	0,71 ± 0.07	0,41 ± 0.04
2,2’-Dichloro-4,4’-methylenedianiline	0,5	0,78 ± 0.06	0,39 ± 0.04
3,3’-Dimethoxybenzidine	0,.5	0,83 ± 0.06	0,22 ± 0.02

C.8.5 Recovery

C.8.5.1 Recovery of 22 soluble aromatic amines

Recovery was determined in a single batch of finger paint containing 22 aromatic amines each spiked at a concentration of 1,0 mg/kg using C.6.2 by two independent laboratories on replicate samples using GC-MS and shown in Table C.10.

Table C.10 — Recovery of 22 primary aromatic amines

Primary aromatic amine	**% Recovery** (Average of Lab 1 and 2)	**Primary aromatic amine**	**% Recovery** (Average of Lab 1 and 2)
o-Toluidine	92	p-Aminoazobenzene	49
4-Methoxy-aniline	98	4,4’-oxydianiline	89
4-Chloroaniline	84	Benzidine	73
6-Methoxy-m-toluidine	76	4,4’-Methylenedianiline	66
2,4,5-Trimethylaniline	67	o-Aminoazotoluene	56
4-Chloro-o-toluidine	99	4,4-Methylenedi-o-toluidine	59
4-Methyl-m-phenylenediamine	< 10	3,3’-Dimethylbenzidine	58
4-Methoxy-m-phenylenediamine	< 10	4,4’-Thiodianiline	58
2-Naphthylamine	44	3,3’-Dichlorobenzidine	106
5-Nitro-o-toluidine	116	2,2’-Dichloro-4,4’-methylene-dianiline	105
4-Aminobiphenyl	64	3,3’-Dimethoxybenzidine	80

C.8.5.2 Verification data

Verification data was determined using C.6.6 (Verification of analytical system) for 22 primary aromatic amines as shown in Table C.11.

Table C.11 — Verification data

Primary aromatic amine	% Recovery	Primary aromatic amine	% Recovery
o-Toluidine	125	p-Aminoazobenzene	24
4-Methoxy-aniline	116	4,4’-Oxydianiline	112
4-Chloroaniline	112	Benzidine	97
6-Methoxy-m-toluidine	113	4,4’-Methylenedianiline	102
2,4,5-Trimethylaniline	102	o-Aminoazotoluene	42
4-Chloro-o-toluidine	107	4,4-Methylenedi-o-toluidine	109
4-Methyl-m-phenylenediamine	58	3,3’-Dimethylbenzidine	96
4-Methoxy-m-phenylenediamine	14	4,4’-Thiodianiline	109
2-Naphthylamine	68	3,3’-Dichlorobenzidine	104
5-Nitro-o-toluidine	117	2,2’-Dichloro-4,4’-methylenedianiline	100
4-Aminobiphenyl	86	3,3’-Dimethoxybenzidine	122
NOTE The data presented is based on a single laboratory.			

C.9 Report

Any report of analysis shall refer to this method and include:

a) precise sample description/identification/article number;

b) type and date of sampling;

c) date of submission and date of analysis;

d) data on procedure (separation and detection);

e) data on quantification procedure;

f) calculated results;

g) a statement as to whether or not a proscribed azo colourant has been detected (see 4.2.2);

h) a statement as to whether the requirements for primary aromatic amines have been met (see 4.5.1);

i) measurement uncertainty (where relevant).

C.10 Additional Information

The effect of evaporation on amine recovery was studied by removing the extracting solvent, *tert*-Butyl methyl ether (MTBE), to dryness at 50 °C. The resulting recoveries were less than 40 % for the target amines, giving an indication of the effects that evaporation to dryness has on amine recovery. The method requires the evaporation of the solvent to approximately 5 ml by rotary evaporation. This volume is transferred to a 10 ml test tube and reduced to a final extract volume of (1 ± 0,01) ml with nitrogen at room temperature.

Due to the polar nature of some amines, clean chromatography conditions are essential when undertaking this analysis.

Annex D
(normative)

List of preservatives allowed for use in finger paints and maximum allowed concentrations

Table D.1 — Preservatives

Ref. No.	Substance	EC Number	CAS Number	Maximum allowed concentration	Limitations and requirements
1	Benzoic acid, sodium benzoate	200–618–2 208–534–8	65–85–0 532–32–1	0,5 % (acid)	
2	Ammonium benzoate calcium benzoate potassium benzoate magnesium benzoate MEA-benzoate methyl benzoate ethyl benzoate propyl benzoate butyl benzoate isobutyl benzoate isopropyl benzoate ‘ phenyl benzoate	217–468–9 218–235–4 209–481–3 209–045–2 224–387–2 202–259–7 202–284–3 219–020–8 205–252–7 204–401–3 213–361–6 202–293–2	1863–63–4 2090–05–3 582–25–2 553–70–8 4337–66–0 93–58–3 93–89–0 2315–68–6 136–60–7 120–50–3 939–48–0 93–99–2	0,5 % (acid)	
3	Propionic acid, ammonium propionate calcium propionate magnesium propionate potassium propionate sodium propionate	201–176–3 241–503–7 223–795–8 209–166–0 206–323–5 205–290–4	79–09–4 17496–08–1 4075–81–4 557–27–7 327–62–8 137–40–6	2 % (acid)	
4	Hexa-2,4-dienoic acid and its salts: Sorbic acid calcium sorbate sodium sorbate potassium sorbate	203–768–7 231–321–6 231–819–3 246–376–1	110–44–1 7492–55–9 7757–81–5 24634–61–5	0,6 % (acid)	
5	Paraformaldehyde		30525–89–4	0,1 % (“free” formaldehyde)	Oral cosmetic products limit
6	Biphenyl-2-ol (o-Phenylphenol) sodium o-phenylphenate potassium o-phenylphenate MEA o-phenylphenate	201–993–5 205–055–6 237–243–9 282–227–7	90–43–7 132–27–4 13707–65–8 84145–04–0	0,2 % expressed as the phenol	
7	Pyrithione zinc	236–671–3	13463–41–7	0,5 %	

[a] For additional labelling requirements for mixtures containing substances with sensitizing properties see GHS (Table 3.4.6).[4] Mixtures containing substances with sensitizing properties in certain concentrations shall be labelled “Contains xxx. May produce an allergic reaction.”

Table D.1 *(continued)*

Ref. No.	Substance	EC Number	CAS Number	Maximum allowed concentration	Limitations and requirements
8	Inorganic sulfites and hydrogen-sulfites: Sodium sulfite ammonium bisulfite ammonium sulfite potassium sulfite potassium hydrogen sulfite sodium bisulfite sodium metabisulfite potassium metabisulfite	231–821–4 233–469–7 233–484–9 233–321–1 231–870–1 231–548–0 231–673–0 240–795–3	7757–83–7 10192–30–0 10196–04–0 10117–38–1 7773–03–7 7631–90–5 7681–57–4 16731–55–8	0,2 % (as “free” SO_2)	
9	Chlorobutanol	200–317–6	57–15–8	0,5 %	
10	4-Hydroxybenzoic acid methylparaben potassium ethylparaben potassium paraben sodium methylparaben sodium ethylparaben ethylparaben sodium paraben potassium methylparaben calcium paraben phenylparaben	99–96–7 99–76–3 36457–19–9 16782–08–4 5026–62–0 35285–68–8 120–47–8 114–63–6 2611–07–2 69959–44–0 17696–62–7	202–804–9 202–785–7 253–048–1 240–830–2 225–714–1 252–487–6 204–399–4 204–051–1 247–464–2 274–235–4 241–698–9	0,4 % (as acid) for single ester, 0,8 % (as acid) for mixtures of esters	
11	3-Acetyl-6-methylpyran-2,4 (3H)-dione and its salts: Dehydroacetic acid sodium dehydroacetate	208–293–9 224–580–1	520–45–6 4418–26–2 16807–48–0	0,6 % (as acid)	
12	Formic acid sodium formate	200–579–1 205–488–0	64–18–6 141–53–7	0,5 % (as acid)	
13	3,3’-Dibromo-4,4’-hexamethylenedioxydi-benzamidine and its salts (including isethionate) (Dibromohexamidine isethionate)	299–116–4	93856–83–8	0,1 %	
14	Undec-10-enoic acid and its salts: Undecylenic acid potassium undecylenate sodium undecylenate calcium undecylenate TEA-undecylenate MEA-undecylenate	203–965–8 222–264–8 215–331–8 282–908–9 260–247–7	112–38–9 6159–41–7 3398–33–2 1322–14–1 84471–25–0 56532–40–2	0,2 % (as acid)	
15	5-Pyrimidinamine, 1,3-bis (2-ethylhexyl)-5- methylhexahydropyrimidin-5-amine (Hexetidine)	205–513–5	141–94–6	0,1 %	
16	2-Bromo-2-nitropropane-1,3-diol (Bronopol)	200–143–0	52–51–7	0,1 %	Not to be used in formulations containing amine substances such as diethanolamine, to avoid generation of nitrosamines
17	2,4-Dichlorobenzyl alcohol	217–210–5	1777–82–8	0,15 %	

[a] For additional labelling requirements for mixtures containing substances with sensitizing properties see GHS (Table 3.4.6).[4] Mixtures containing substances with sensitizing properties in certain concentrations shall be labelled “Contains xxx. May produce an allergic reaction.”

Table D.1 *(continued)*

Ref. No.	Substance	EC Number	CAS Number	Maximum allowed concentration	Limitations and requirements
18	1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl) urea (Triclocarban)	202–924–1	101–20–2	0,2 %	Purity criteria:3,3′,4,4′-Tetrachloro-azobenzene < 1 ppm 3,3′,4,4′-Tetrachloro-azoxybenzene < 1 ppm
19	5-Chloro-2- (2,4- dichlorophenoxy) phenol (Triclosan)	222–182–2	3380–34–5	0,3 %	
20	Chloroxylenol	201–793–8	88–04–0	0,5 %	
21	N,N″-methylenebis[N′-[3-(hydroxymethyl)-2,5-dioxoimidazolidin-4-yl]urea] (Imidazolidinyl urea)	254–372–6	39236–46–9	0,6 %	
22	Poly(methylene),.alpha.,.omega.-bis[[[(aminoiminomethyl) amino]iminomethyl] amino]-, dihydrochloride (Polyaminopropyl biguanide)		70170–61–5 28757–47–3 133029–32–0	0,3 %	
23	2-Phenoxyethanol	204–589–7	122–99–6	1,0 %	
24	Methenamine	100–97–0	202–905–8	0,15 %	
25	Methenamine-3-chloro-allylochloride (Quaternium-15)	223–805–0	4080–31–3	0,2 %	
26	1-(4-Chlorophenoxy)-1-(imidazol-1-yl)-3,3-dimethylbutan-2-one (Climbazole)	253–775–4	38083–17–9	0,5 %	
27	1,3-Bis (hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione (DMDM Hydantoin)	229–222–8	6440–58–0	0,6 %	
28	1-Hydroxy-4-methyl-6-(2,4,4-trimethylpentyl) 2-pyridon and its monoethanolamine salt (Piroctone Olamine)	272–574–2	50650–76–5 68890–66–4	0,5 %	
29	2,2′-methylenebis(6-bromo-4-chlorophenol) (Bromochlorophene)	239–446–8	15435–29–7	0,1 %	
30	4-Isopropyl-m-cresol (o-Cymen-5-ol)	221–761–7	3228–02–2	0,1 %	
31	Mixture of 5-Chloro-2-methyl-isothiazol-3(2H)-one and 2-methylisothiazol-3(2H)-one with magnesium chloride and magnesium nitrate	247–500–7	26172–55–4 2682–20–4 55965–84–9	0,000 8 % (of a mixture in the ratio 3:1 of 5-chloro-2-methylisothiazol 3(2H)-one and 2-methylisothiazol-3 (2H)-one	See [a].
32	2-methylisothiazol-3(2H)-one (MIT)	220–239–6	2682–20–4	0,01 %	See [a].

[a] For additional labelling requirements for mixtures containing substances with sensitizing properties see GHS (Table 3.4.6).[4] Mixtures containing substances with sensitizing properties in certain concentrations shall be labelled “Contains xxx. May produce an allergic reaction.”

Table D.1 *(continued)*

Ref. No.	Substance	EC Number	CAS Number	Maximum allowed concentration	Limitations and requirements
33	2-Benzyl-4-chlorophenol (Chlorophene)	204–385–8	120–32–1	0,2 %	
34	2-Chloroacetamide	201–174–2	79–07–2	0,3 %	
35	N,N″-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediamidine and its digluconate, diacetate and dihydrochloride: Chlorhexidine Chlorhexidine Diacetate Chlorhexidine Digluconate Chlorhexidine Dihydrochloride	200–238–7 200–302–4 242–354–0 223–026–6	55–56–1 56–95–1 18472–51–0 3697–42–5	0,3 % (as chlorhexidine)	
36	Alkyl (C 12–22) trimethyl ammonium bromide and chloride: Behentrimonium chloride cetrimonium bromide cetrimonium chloride laurtrimonium bromide laurtrimonium chloride steartrimonium bromide steartrimonium chloride	241–327–0 200–311–3 203–928–6 214–290–3 203–927–0 214–294–5 203–929–1	17301–53–0 57–09–0 112–02–7 1119–94–4 112–00–5 1120–02–1 112–03–8	0,1 %	
37	4,4-Dimethyl-1,3-oxazolidine	257–048–2	51200–87–4	0,1 %	The pH of the finished product may not be lower than 6.
38	N-(Hydroxymethyl)-N-(dihydroxymethyl-1,3-dioxo-2,5-imidazolidinyl-4)-N′-(hydroxymethyl) urea (Diazolidinyl Urea)	278–928–2	78491–02–8	0,5 %	
39	Benzenecarboximidamide, 4,4′-(1,6-hexanediyl-*bis*-(oxy))-*bis*-(3-nitrobenzamidine) and its salts (including isethionate and phydroxybenzoate): hexamidine, hexamidine diisethionate, hexamidine paraben	211–533–5 299–055–3	3811–75–4 659–40–5 93841–83–9	0,1 %	
40	Pentane-1,5-dial (Glutaraldehyde, Glutaral)	203–856–5	111–30–8	0,1 %	
41	3-(p-Chlorophenoxy)-propane-1,2 diol (Chlorphenesin)	203–192–6	104–29–0	0,3 %	
42	Sodium N-hydroxymethyl-glycinate	274–357–8	70161–44–3	0,5 %	
43	Benzenemethanaminium, N,N-dimethyl-N-[2-[2-[4-(1,1,3,3,-tetramethylbutyl)phenoxy] ethoxy] ethyl]-, chloride (Benzethonium Chloride)	204–479–9	121–54–0	0.1 %	

[a] For additional labelling requirements for mixtures containing substances with sensitizing properties see GHS (Table 3.4.6).[4] Mixtures containing substances with sensitizing properties in certain concentrations shall be labelled “Contains xxx. May produce an allergic reaction.”

Annex E
(normative)

Method for the determination of hexachlorobenzene, polychlorinated biphenyls and benzo[α]pyrene

E.1 Principle

Solvent extractable benzo[α]pyrene (B[α]P), hexachlorobenzene (HCB) and polychlorinated biphenyl (PCB) congeners are determined in finger paints by mixing with anhydrous sodium sulfate and extracting with either a 1:1 mixture of cyclohexane and acetone for HCB and PCBs or 2:1 mixture of toluene and acetone for B[α]P using a soxhlet extractor. The extract is cleaned up and concentrated prior to analysis using gas chromatography with a mass spectrometric detection (GC-MS) using the internal standard method of calibration.

E.2 Standards, reagents and solvents

Reagent-grade chemicals are to be used, unless otherwise specified.

The stability of all calibration solutions shall be checked regularly. These should be stable for up to six months when stored in the dark at (5 ± 2) °C.

E.2.1 Hexane, analytical grade.

E.2.2 Cyclohexane, analytical grade.

E.2.3 Acetone, analytical grade.

E.2.4 2,2,4-trimethylpentane, analytical grade.

E.2.5 Toluene, analytical grade.

E.2.6 Cyclohexane (E.2.2): **acetone** (E.2.3) 1:1 v/v mixture.

E.2.7 Toluene (E.2.5):**acetone** (E.2.3) 2:1 v/v mixture.

E.2.8 Anhydrous sodium sulfate.

E.2.9 Standards.

E.2.9.1 Hexachlorobenzene (HCB), CAS No 118-74-1, > 99 %.

E.2.9.2 Commercial PCB standard mixture or individual PCB congeners:

- PCB congener 11 (3,3’-Dichlorobiphenyl), CAS No: 2050-67-1;
- PCB congener 28 (2,4,4’-Trichlorobiphenyl), CAS No: 7012-37-5;
- PCB congener 52 (2,2’,5,5’-Tetrachlorobiphenyl), CAS No: 35693-99-3;

- PCB congener 101 (2,2',4,5,5'-Pentachlorobiphenyl), CAS No: 37680-73-2;
- PCB congener 118 (2,3',4,4',5-Pentachlorobiphenyl), CAS No: 31508-00-6;
- PCB congener 138 (2,2',3,4,4',5'-Hexachlorobiphenyl), CAS No: 35065-28-2;
- PCB congener 153 (2,2',4,4',5,5'-Hexachlorobiphenyl), CAS No: 35065-27-1;
- PCB congener 180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl), CAS No:35065-29-3;
- PCB congener 209 (Decachlorobiphenyl), CAS No: 2051-24-3,

E.2.9.3 Benzo[α]pyrene (B[α]P), CAS No: 50-32-8, > 99 %.

E.2.10 Internal standards.

E.2.10.1 HCB 13C6, CAS No: 118-74-1.

E.2.10.2 PCB congener 101 13C12, CAS No: 37680-73-2.

E.2.10.3 PCB congener 138 13C12, CAS No: 35065-28-2.

E.2.10.4 B [α]P d12, CAS No: 63466-71-7.

E.2.11 Primary standard solutions.

E.2.11.1 Prepare a 100 mg/l HCB primary standard solution (E.2.9.1) in 2,2,4-trimethylpentane (E.2.4).

E.2.11.2 Prepare a primary standard solution (E.2.9.2) containing the nine PCB congeners each at a concentration of 100 mg/l in 2,2,4-trimethylpentane (E.2.4).

E.2.11.3 Prepare a 200 mg/l B[α]P primary standard solution (E.2.9.3) in 2,2,4-trimethylpentane (E.2.4).

E.2.12 Internal standard solutions.

E.2.12.1 HCB $^{13}C_6$.

Prepare a stock solution of internal standard (E.2.10.1) at 10 mg/l in 2,2,4-trimethylpentane (E.2.4).

E.2.12.2 PCB congener 101 $^{13}C_{12}$ and PCB congener 138 $^{13}C_{12}$.

Prepare a stock solution of each internal standard (E.2.10.2) at 10 mg/l in 2,2,4-trimethylpentane (E.2.4).

E.2.12.3 PCB congener 138 $^{13}C_{12}$.

Prepare a stock solution of each internal standard (E.2.10.3) at 10 mg/l in 2,2,4-trimethylpentane (E.2.4).

E.2.12.4 B[α]P d_{12}.

Prepare a stock solution of internal standard (E.2.10.4) at 1 mg/l in 2,2,4-trimethylpentane (E.2.4).

E.2.13 Calibration standards.

E.2.13.1 HCB and PCB congeners calibration standards.

Prepare six calibration solutions in the concentration range 0,02 mg/l to 1 mg/l by dilution of the primary standard HCB (E.2.11.1) and PCB mixed congener (E.2.11.2) standard solutions in 2,2,4-trimethylpentane (E.2.4). Each calibration solution shall also contain 0,5 mg/l of the HCB $^{13}C_6$ (E.2.12.1), 0,25 mg/l PCB congener 101 $^{13}C_{12}$ (E.2.12.2) and PCB congener 138 $^{13}C_{12}$ (E.2.12.3) internal standards.

E.2.13.2 B[α]P calibration standard.

Prepare six calibration solutions in the concentration range 0,005 mg/l to 0,02 mg/l by dilution of the primary B[α]P (E.2.11.3) standard solution in 2,2,4-trimethylpentane. Each calibration solution shall also contain 0,02 mg/l of the B[α]P d_{12} (E.2.12.4) internal standard.

E.2.14 Recovery solutions.

E.2.14.1 HCB recovery solution

Prepare a recovery solution containing 10 mg/l of HCB in 2,2,4-trimethylpentane (E.2.4).

E.2.14.2 Mixed PCB congener recovery solution

Prepare a recovery solution containing 1 mg/l of each polychlorinated biphenyl congener in 2,2,4-trimethylpentane (E.2.4).

E.2.14.3 B[α]P recovery solution

Prepare a recovery solution containing 0,1 mg/l of B[α]P in 2,2,4-trimethylpentane (E.2.4).

E.3 Apparatus

Standard laboratory glassware and equipment and the following shall be used. Apparatus should be free from contamination before use. Glassware should be rinsed with acetone and then hexane before use and allowed to drain.

E.3.1 Amber coloured glass bottle approximately 40 ml volume with tight-fitting screw cap.

E.3.2 Analytical balance, capable of weighing to 4 decimal places.

E.3.3 Glass microfibre thimble, 33 mm diameter x 100 mm.

E.3.4 Soxhlet extractor with siphon cup to hold a 33 mm diameter x 100 mm thimble.

E.3.5 Water cooled condenser.

E.3.6 250 ml round bottom flask.

E.3.7 Spark proof heating mantle.

E.3.8 Sample concentration system with nitrogen gas stream.

E.3.9 Solid phase extraction column made from either glass or polypropylene, 25 mm to 30 mm internal diameter, 140 mm to 150 mm length, filled with about 20 g porous, granular “kieselguhr” SPE material (or commercial SPE column).

E.4 Instrumentation

E.4.1 A gas chromatograph-mass spectrometric system fitted with a capillary column and glass-lined injector, capable of operating in electron impact mode with selected ion monitoring which permits different groups of ions to be monitored at selected time intervals during the analysis.

E.4.2 Analytical capillary column, for example HT-8, 50 m length x 0,22 mm ID x 0,25 mm film thickness 8 % phenyl polysiloxane-carborane or equivalent.

E.5 Sampling

The finger paint is mixed vigorously using a glass rod to ensure a homogeneous test portion can be sampled.

Finger paints are mainly water based and so an immediate test portion should be taken once a container has been opened.

It is important to take care when repeating sampling over time from a container as the composition of the test portion may vary through loss of constituents to the atmosphere.

E.6 Procedure

E.6.1 General

The sample preparation is the same for all analytes using the solvent and solid phase extraction stages. However, to achieve the lower limits specified for B[α]P the concentration stage has been separately described to HCB and PCB congeners.

E.6.2 Sample preparation

A screw cap amber bottle (E.3.1) is filled with approximately 6 g of anhydrous sodium sulfate (E.2.8). Weigh accurately (1,0 ± 0,1) g of the test portion onto the surface of the anhydrous sodium sulfate and record the weight (M). Add an additional 6g of anhydrous sodium sulfate to cover the test portion and tightly close the screw cap.

Shake the bottle vigorously for (60 ± 5) s to mix the test portion and sodium sulfate. Remove the cap and allow the mixture to stand for 24 h ± 1 h under standard laboratory conditions ensuring the mixture will not come into contact with any possible contaminants.

After 24 h, replace the screw cap onto the amber bottle and shake vigorously again for a further (60 ± 5) s.

The mixture should consist of particles no larger than 1 mm to 5 mm, where larger particles have formed a glass rod can be used to break up the larger particles.

E.6.3 Solvent extraction of soluble HCB, PCBs and B[α]P

E.6.3.1 Solvent extraction of soluble HCB and PCBs

Transfer the mixture in E.6.2 into a glass microfibre thimble (E.3.3) and add a filter paper disc to the top of the thimble. Insert the thimble into a soxhlet extractor (E.3.4) and connect to a water cooled condenser (E.3.5). Add approximately 175 ml of cyclohexane/acetone (E.2.6) into a 250 ml round bottom flask (E.3.6) and connect to the soxhlet extractor, place on a heating mantle (E.3.7) and reflux gently for > 6 h.

Allow sufficient time for the cyclohexane/acetone to cool before disconnecting the soxhlet extractor and gently evaporate the cyclohexane/acetone extract to approximately 5-10 ml using a rotary evaporator or equivalent sample concentration system (E.3.8).

Transfer the cyclohexane/acetone extract into a graduated glass tube washing with 2 × 3 ml cyclohexane and evaporate the extract to approximately 3 ml under a gentle stream of nitrogen (E.3.8).

E.6.3.2 Solvent extraction of soluble B[α]P

Transfer the mixture in E.6.2 into a glass microfibre thimble (E.3.3) and add a filter paper disc to the top of the thimble. Insert the thimble into a soxhlet extractor (E.3.4) and connect to a water cooled condenser (E.3.5). Add approximately 200 ml of toluene/acetone (E.2.7) into a 250 ml round bottom flask (E.3.6) and connect to the soxhlet extractor, place on a heating mantle (E.3.7) and reflux gently for > 6 h.

Allow sufficient time for the toluene/acetone to cool before disconnecting the soxhlet extractor and gently evaporate the toluene/acetone extract to approximately 3 ml using a rotary evaporator or equivalent sample concentration system (E.3.8).

Transfer the toluene/acetone extract into a graduated glass tube washing with 1 ml toluene and evaporate the extract to approximately 3ml under a gentle stream of nitrogen (E.3.8).

E.6.4 Solid phase extraction

E.6.4.1 Solid phase extraction of HCB and PCBs

Transfer the extract obtained in E.6.3 onto a column containing kieselguhr (E.3.9) and leave to stand for approximately 1 h. Then wash the column with 3 × 5 ml portions of cyclohexane (E.2.2) collecting the eluent into a suitable glass vessel. To the glass vessel and eluent add approximately 6 ml of 2,2,4-trimethylpentane (E.2.4).

E.6.4.2 Solid phase extraction of B[α]P

Transfer the extract obtained in E.6.3.2 onto a column containing kieselguhr (E.3.9) and leave to stand for approximately 1 h. Then wash the column with 3 × 5 ml portions of toluene (E.2.5) collecting the eluent into a suitable glass vessel.

E.6.5 Sample concentration for determining HCB and PCB congeners

Evaporate the eluent obtained in E.6.4 to approximately 3 ml using the sample concentrator (E.3.8) and quantitatively transfer into a graduated test tube and fill to the 6 ml mark with 2,2,4-trimethylpentane. Transfer 1 ml to a sample vial and add 0,025 ml of the HCB internal standard solution (E.2.12.1) and 0,05 ml of each PCB internal standard solutions (E.2.12.2 and E.2.12.3) for quantitative determination by GC-MS.

E.6.6 Sample concentration for determining B[α]P

Evaporate the eluent obtained in E.6.4 to approximately 3 ml using the sample concentrator (E.3.8) and quantitatively transfer into a graduated test tube and fill to the 4 ml mark with 2,2,4-trimethylpentane. Transfer 1 ml to a sample vial and add 0,05 ml of the B[α]P internal standard solution (E.2.12.4) for quantitative determination by GC-MS.

E.6.7 Gas chromatography conditions

Set up the gas chromatography-mass spectrometer detection system according to manufacturer's instructions, to monitor the selected mass fragmentation ions for HCB, PCB congeners, and B[α]P.

The following conditions have been shown to be suitable.

Capillary column	HT-8 or equivalent type, length: 50 m, internal diameter: 0,25 mm, film thickness: 0,22 µm
Injector	split/splitless
Injection temperature	220 °C
Carrier	Helium
Injection volumes	1,0 µl, split 1:15
Detection	Mass spectrometry
Temp. programme for HCB and PCB congeners	60 °C (hold 2 min), 60 °C to 170 °C (3,5 °C/min), 300 °C (12 min)
Temp. Programme for B[α]P	60 °C (hold 1 min), 60 °C to 170 °C (30 °C/min), (hold 1 min), 170 to 300 °C (20-30 °C/min), (hold 25 min)

E.6.8 Mass spectrometry

A single quadrupole MS instrument is recommended with ionization by electronic impact at 70 eV.

E.6.9 SIM mode

E.6.9.1 General

In SIM mode, the fragment ions allow quantification by using one of the three ions as the target quantification ion and the remaining two ions as qualifiers identified in Tables E.1 and E.2.

NOTE T1 is considered as the target ion for quantification.

The time spent on each ion (dwell time) shall be the same for all ions within a given window. The m/z values are rounded. Exact values should be used as SIM parameters.

Table E.1 — List of ions for quantification

Component	Target ion (m/z)	Qualifier 1 (m/z)	Qualifier 2 (m/z)
HCB	284	249	286
PCB 11	222	224	152
PCB 28	256	258	186
PCB 52	292	290	294
PCB 101	326	328	324
PCB-118	326	328	324
PCB-153	360	362	290
PCB 138	360	362	290
PCB 180	396	394	324
PCB 209	498	500	496
HCB $^{13}C_6$	294		
PCB 101 $^{13}C_{12}$	338		
PCB 138 $^{13}C_{12}$	372		

Table E.2 — List of ions for quantification

Component	Target ion (m/z)	Qualifier 1 (m/z)	Qualifier 2 (m/z)
B(α)P	255	250	253
B(α) d12	264		

E.6.9.2 Maximum permitted tolerances

The relative intensities of the ion for quantification against respectively the two qualifiers, expressed as a percentage of the intensity of the most intense ion, shall correspond to those of the calibration standard solutions, at comparable concentrations, measured under the same conditions, within the tolerances described Table E.3.

The calibration standard used as reference should be at the middle of the calibration curve.

Table E.3 — Maximum permitted tolerances for relative ion intensities

Relative intensity % of base ion intensity	Relative range of the response
> 50 %	±10 %
> 20 % - 50 %	±15 %
> 10 % - 20 %	±20 %
≤ 10 %	±50 %

Figure E.1 shows an example of chromatographic separation of HCB and 9 PCB Congeners.

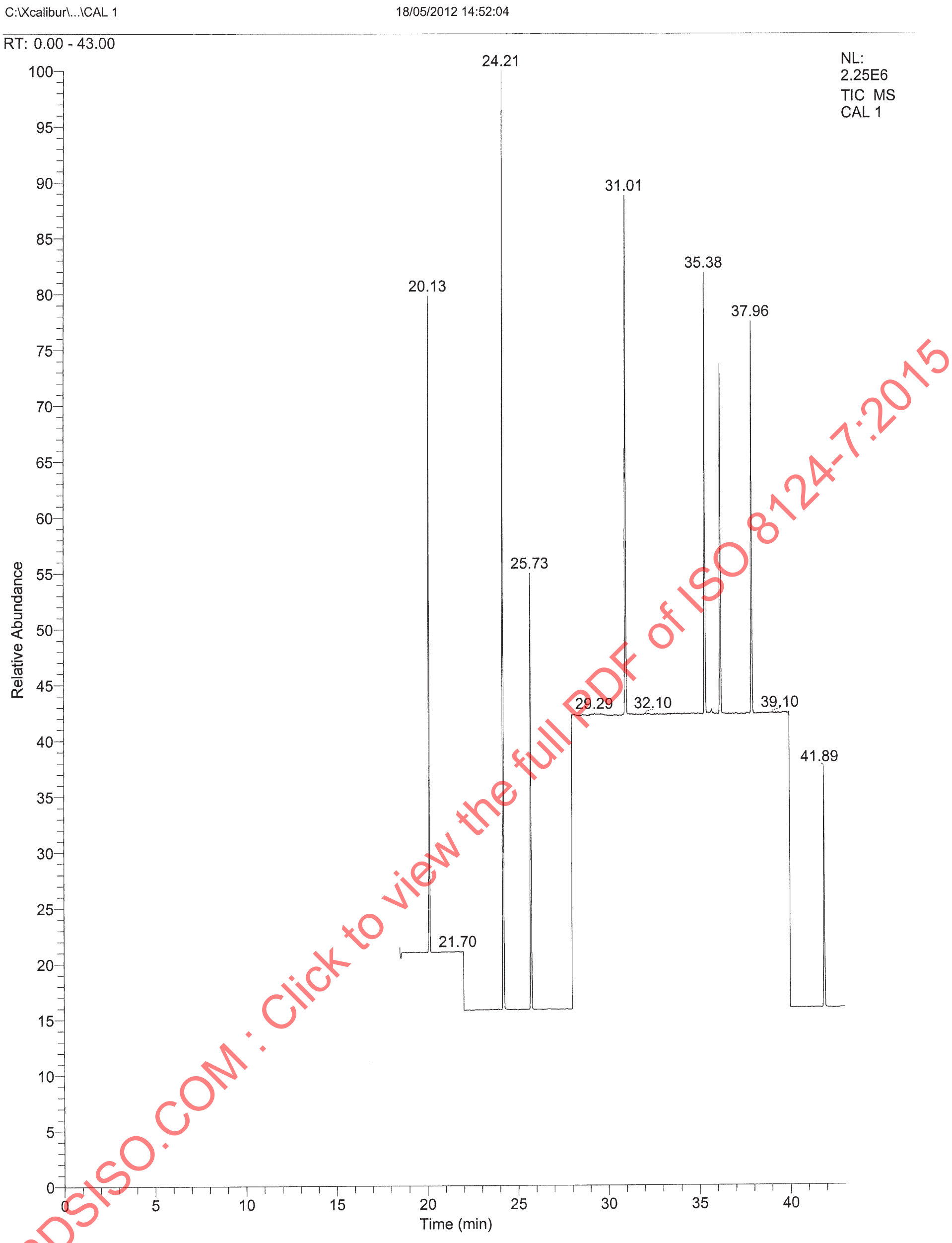

Figure E.1 — Example of chromatographic separation of HCB and 9 PCB Congeners

Table E.4 shows a key to retention time for HCB and 9 PCB Congeners.

Table E.4 — Key to retention time for HCB and 9 PCB Congeners

Component	Key retention time (min)
HCB	19,65
PCB 11	20,76
PCB 28	23,57
PCB 52	25,03
PCB 101	30,14
PCB 118	34,43
PCB 153	35,28
PCB 138	36,98
PCB 180	40,87
PCB 209	49,00

Figure E.2 shows an example of chromatographic separation of B[α]P and B[α]P d12.

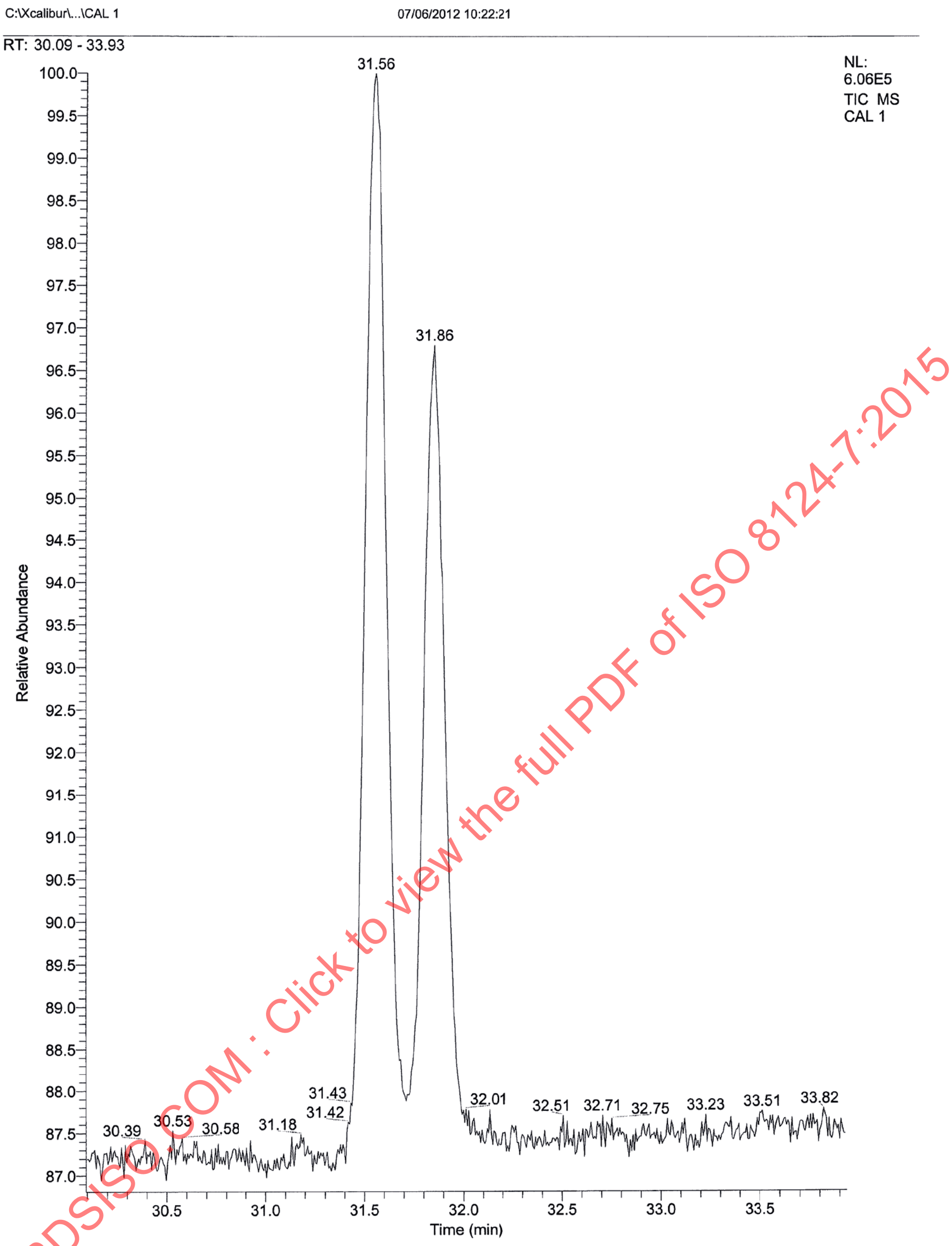

Figure E.2 — Example of chromatographic separation of B[α]P and B[α]P d12

Table E.5 shows a key to retention time for benzo[α]pyrene and benzo[α]pyrene d12.

Table E.5 — Key to retention time for benzo[α]pyrene and benzo[α]pyrene d12

Component	Key retention time min
B[α]P	31,86
B[α]P d12	31,56

E.6.9.3 Verification of analytical system

To check the analytical procedure for HCB, add 0,5 ml of a recovery solution (E.2.14.1) onto the sodium sulfate described in E.2.8 and follow the procedure E.6.2 to E.6.4 .

To check the analytical procedure for PCB congeners, add 0,3 ml of a recovery solution (E.2.14.2) onto the sodium sulfate described in E.2.8 and follow the procedure E.6.2 to E.6.4.

The recovery rate of the hexachlorobenzene and the PCB congeners should be at least 70 %.

To check the analytical procedure for B[α]P, add 0,2 ml of a recovery solution (E.2.14.3) onto the sodium sulfate described in E.2.8 and follow the procedure E.6.2 to E.6.4.

The recovery rate of B[α]P shall be at least 70 %.

E.7 Calculation

E.7.1 For HCB, PCB congeners and B[α]P determination, the internal standard calibration method is used. An *R* value is determined, which is the ratio between the intensity from the ion used for quantification and the internal standard ion. Prepare a calibration curve by plotting the concentration of HCB, PCB and B[α]P calibration solutions against the corresponding *R* value. Determine the concentration *C* of each analyte in mg/l in the sample solution directly from the calibration curve.

E.7.2 The concentration of PCBs in the original sample at each level of chlorination is given by:

$$C_{LC} = \frac{C}{M} \cdot Vmgkg^{-1} \qquad \text{(E.1)}$$

where

C_{LC}	is the concentration of PCB congeners at each level of chlorination (where n is 1 to 7) in the sample, in mg/kg;
M	is the mass of sample extracted, in g;
V	is the volume of sample solution, in ml;
C	is the concentration PCB congener in sample solution, in mg/l.

$$C_{Total} = \sum_{1}^{7} C_{Lc} \qquad \text{(E.2)}$$

where

C_{Total}	is the concentration of all PCB congeners determined in the original sample, in mg/kg;
C_{LC}	is the concentration of PCB congeners at each level of chlorination (where n is 1 to 7) in the sample, in mg/kg.

E.7.3 The concentration of HCB in the original sample is given by:

$$C_{HCB} = \frac{C}{M} \cdot Vmgkg^{-1} \qquad \text{(E.3)}$$

where

C_{HCB}	is the concentration of HCB in the original sample, in mg/kg;

M is the mass of sample extracted, in g;

V is the volume of sample solution, in ml;

C is the concentration HCB in sample solution, in mg/l.

E.7.4 The concentration of B[α]P in the original sample is given by:

$$C_{B[\alpha]P} = \frac{C}{M} \cdot Vmgkg^{-1} \qquad \text{(E.4)}$$

where

$C_{\mathrm{B[\alpha]P}}$ is the concentration of B[α]P in the original sample, in mg/kg;

M is the mass of sample extracted, in g;

V is the volume of sample solution, in ml;

C is the concentration B[α]P in sample solution, in µg/ml.

The B[α]P concentration is calculated from the area of the B[α]P peak and is given as mass portion *w*, in mg/kg, of B[α]P in test material according to Formula (E.5):

$$w = \frac{A_S \cdot C_C \cdot V_S}{A_C \cdot E_S} \qquad \text{(E.5)}$$

where

w is the mass portion of single amine component in test material, in mg/kg;

A_S is the peak area of the amine in the sample solution, in area units;

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

C_C is the concentration of the amine in the calibration solution (µg/ml);

V_S is the volume of test solution obtained in C.6.4 used for chromatographic analysis, in ml;

E_S is the initial mass of sample in the end volume, in g.

If an internal standard has been used, the mass portion of amine component (w) is given as:

$$w = \frac{A_{IS(S)}}{A_{IS(C)}} \qquad \text{(E.6)}$$

where

w is the mass portion of B[α]P;

$A_{IS(S)}$ the peak area of the internal standard in the sample solution in area units;

$A_{IS(C)}$ is the peak area of the internal standard in the calibration solution in area units.

E.8 Precision

E.8.1 Linearity

The correlation coefficient shall be > 0,990.