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BSI Standards Publication

Food analysis — Determination of benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by high performance liquid chromatography with fluorescence detection (HPLC-FD)

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National foreword

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

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Food analysis - Determination of benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by high performance liquid chromatography with fluorescence detection (HPLC-FD)

Analyse des produits alimentaires - Dosage du benzo(a)pyrène, benzo(a)anthracène, chrysène et benzo(b)fluoranthène dans les denrées alimentaires par chromatographie en phase liquide à haute performance avec détection de fluorescence (HPLC-FD)

Lebensmittelanalytik - Bestimmung von Benzo[a]pyren, Benz[a]anthracen, Chrysen und Benzo[b]fluoranthen in Lebensmitteln mittels Hochleistungs-Flüssigkeitschromatographie mit Fluoreszenzdetektion (HPLC-FD)

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Foreword

This document (CEN/TS 16621:2014) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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1 Scope

This Technical Specification specifies a method for the determination of benzo[*a*]pyrene (BaP) plus benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF) and chrysene (CHR) in several food matrices. The method is based on size exclusion chromatography (SEC) cleanup, followed by quantification with high performance liquid chromatography (HPLC) with programmable fluorescence detection. This method has been in-house validated via the analysis of spiked samples of edible olive oil, fresh mussels, smoked fish, smoked meat products, processed cereal-based foods for young children, infant formulae, chocolate and food supplements (isoflavones) at levels ranging from 0,25 µg/kg to 1,00 µg/kg and from 4,95 µg/kg to 23,53 µg/kg, depending on the Polycyclic Aromatic Hydrocarbon (PAH) or the matrix. This method complies with the performance characteristics specified for BaP, BaA, BbF and CHR in current legislation [3].

The method has been shown to be applicable to a variety of additional matrices as meat products, fresh fish, paprika, roasted coffee, bread, herbs, breakfast cereals, beer, sunflower oil, olives and fried tomato, with a limit of quantification below 0,5 µg/kg.

In addition, the method was tested in-house and shown to be applicable also for the quantification of the other 12 PAHs of the 15+1 EU priority PAHs set (benzo[*c*]fluorene (BcL), benzo[*j*]fluoranthene (BjF), benzo[*k*]fluoranthene (BkF), cyclopenta[*cd*]pyrene (CPP), dibenz[*a,h*]anthracene (DhA), dibenzo[*a,e*]pyrene (DeP), benzo[*ghi*]perylene (BgP), dibenzo[*a,h*]pyrene (DhP), dibenzo[*a,i*]pyrene (DiP), dibenzo[*a,l*]pyrene (DlP), indeno[1,2,3-*cd*]pyrene (IcP), 5-methylchrysene (5MC)) in all matrices listed above and at similar level ranges, except for CPP, where a UV detection had to be used with limits of quantification above 8 µg/kg.

For the determination of PAHs in edible fats and oils, two other standards are also available, EN ISO 22959 and EN ISO 15753 (see [1] and [2]).

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.

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

The PAHs are extracted from solid matrices with dichloromethane. In case of edible oils, the samples are simply dispersed in dichloromethane. Aliquots of crude extracts in dichloromethane are purified by SEC. The final extracts are analysed by HPLC under gradient conditions with programmable fluorescence detection.

4 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis. For storing and expiring dates of use of substances and commercially available solutions, supplier indications or certificates shall be followed. Refrigerated standard solutions shall reach room temperature before being used.

WARNING 1 — Some PAHs are considered carcinogenic. Persons using this document should be familiar with normal laboratory practices. It is the responsibility of the user of this document to apply practices which are in agreement with applicable occupational safety and health practices.

WARNING 2 — Dispose chemical waste according to applicable environmental rules and regulations.

WARNING 3 — PAHs are degraded by UV light. Protect PAHs solutions from light (keep in the dark, use aluminium foil or amber glassware).

WARNING 4 — The analyst shall ensure that samples do not become contaminated during sample preparation. Containers shall be rinsed with high purity acetone or hexane before use to minimize the risk of contamination. Wherever possible, apparatus and equipment coming into contact with the sample shall be made of inert materials such as aluminium, glass or polished stainless steel. Some precaution is needed when using plastics as polypropylene or PTFE because the analytes may be adsorbed onto these materials.

4.1 Helium purified compressed gas (purity equivalent to 99,995 % or better). For solvent degassing, if needed.

4.2 Nitrogen purified compressed gas (purity equivalent to 99,995 % or better).

4.3 Acetone.

4.4 *n*-Hexane.

4.5 Dichloromethane.

4.6 Acetonitrile.

4.7 Methoxychlor.

4.8 Perylene.

4.9 Sulfur.

4.10 Corn oil, commercial.

4.11 HPLC mobile phase solvent A: Water.

The mobile phase solvent A should be degassed.

4.12 HPLC mobile phase solvent B: Acetonitrile (4.6).

The mobile phase solvent B should be degassed.

4.13 Cyclohexane.

4.14 Ethyl acetate.

4.15 Mixture of cyclohexane and ethyl acetate.

Mix one part per volume of cyclohexane (4.13) with one part per volume of ethyl acetate (4.14).

4.16 Anhydrous sodium sulphate.

4.17 Polycyclic aromatic hydrocarbons.

4.17.1 Benzo[*a*]pyrene (BaP).

4.17.2 Chrysene (CHR).

4.17.3 Benzo[*b*]fluoranthene (BbF).

4.17.4 Benz[*a*]anthracene (BaA).

4.17.5 Benzo[*k*]fluoranthene (BkF).

4.17.6 Dibenzo[*a,h*]anthracene (DhA).

4.17.7 Benzo[*g,h,i*]perylene (BgP).

4.17.8 Indeno[1,2,3-*cd*]pyrene (IcP).

4.17.9 Benzo[*c*]fluorene (BcL).

4.17.10 Cyclopenta[*c,d*]pyrene (CPP).

4.17.11 5 – Methylchrysene (5MC).

4.17.12 Benzo[*j*]fluoranthene (BjF).

4.17.13 Dibenzo[*a,l*]pyrene (DlP).

4.17.14 Dibenzo[*a,e*]pyrene (DeP).

4.17.15 Dibenzo[*a,i*]pyrene (DiP).

4.17.16 Dibenzo[*a,h*]pyrene (DahP).

4.17.17 15+1 EU priority PAHs standard solution containing 10 µg/ml each, in appropriate organic solvent, preferably acetonitrile.

4.18 PAH4 standard solution

Prepare a standard solution of 10 µg/ml PAH4 in acetonitrile by weighing carefully the proper amounts of the 4 PAHs (BaP, BaA, BbF and CHR) individually in acetonitrile. Store this solution under refrigeration conditions. A solution stored in this way is stable for at least 12 months. If longer stability is proven, the solution can still be applied.

4.19 PAH4 stock solution

Prepare a stock solution of 500 ng/ml in acetonitrile by diluting exactly 500 µl of the 10 µg/ml PAH4 standard solution (4.18) to 10 ml with acetonitrile (4.6) into a calibrated 10 ml volumetric flask. Store this solution under refrigeration conditions. A solution stored in this way is stable for at least 12 months. If longer stability is proven, the solution can still be applied.

4.20 PAH4 working solution

Prepare a working solution of 50 ng/ml in acetonitrile, by diluting 1 ml of the 500 ng/ml PAH4 stock solution in acetonitrile (4.19) up to 10 ml with acetonitrile (4.6) into a calibrated 10 ml volumetric flask. Store this solution under refrigeration conditions. A solution stored in this way is stable for at least six months. If longer stability is proven, the solution can still be applied.

4.21 15+1 PAHs stock solution

Prepare a stock solution of 500 ng/ml in acetonitrile by pipetting exactly 500 µl of the 10 µg/ml 15+1 PAHs standard solution (4.17.17) into a calibrated 10 ml volumetric flask. Take them to dryness by evaporation under nitrogen, and redissolve in 10 ml of acetonitrile (4.6). Store this solution under refrigeration conditions. A solution stored in this way is stable for at least 12 months. If longer stability is proven, the solution can still be applied.

In case of commercial availability of 15+1 EU priority PAHs standard solution containing 10 µg/ml each, in acetonitrile, the 15+1 PAHs stock solution can be prepared directly by diluting 500 µl of that solution up to 10 ml with acetonitrile.

4.22 15+1 PAHs working solution

Prepare a working solution of 50 ng/ml in acetonitrile, by diluting 1 ml of the 500 ng/ml 15+1 PAHs stock solution in acetonitrile (4.21) to 10 ml with acetonitrile into a calibrated 10 ml volumetric flask. Store this solution under refrigeration conditions. A solution stored in this way is stable for at least six months. If longer stability is proven, the solution may still be applied.

5 Apparatus

Usual laboratory glassware and equipment and, in particular, the following:

5.1 High speed blender, for solid samples and **vortex mixer** for liquid samples.

5.2 Filter papers, suitable for qualitative analysis, prefolded.

5.3 Round bottomed glass tubes, of 250 ml capacity, suitable for the high speed blender.

5.4 Evaporator, with water bath and flushing nitrogen capability.

5.5 Analytical balance, accuracy to the nearest 0,000 1 g.

5.6 Laboratory balance, accuracy to the nearest 0,1 g.

5.7 Glass syringe, of 5 ml capacity.

5.8 Microsyringes, of 250 µl, 500 µl and 1 000 µl capacity.

5.9 Calibrated volumetric flasks, of 5 ml and 10 ml capacity.

5.10 Displacement pipettes, of 200 µl, with appropriate tips.

5.11 Glass vials, approximately 1,8 ml capacity and crimp caps.

5.12 Graduated pipette, of 5 ml capacity.

5.13 Size-Exclusion Chromatography (SEC) system, comprising the following:

5.13.1 HPLC pump (isocratic), capable of pumping 5 ml/min pulse free.

5.13.2 Injection system, suitable for 1,0 ml and 0,2 ml injection volume.

5.13.3 Two SEC cleanup columns, 19 mm x 150 mm and 19 mm x 300 mm, connected in series, packed with high-performance, fully porous, highly cross-linked, styrene divinylbenzene copolymer particles, 10 nm pore size with nominal particle size of 15 µm.

5.13.4 UV detector, capable to provide λ = 254 nm.

5.13.5 Fraction collector.

5.13.6 Recorder, integrator or computer based data processing system.

5.13.7 Calibration

Connect the columns to the system and calibrate the SEC cleanup columns following the instructions of the manufacturer. The calibration solution requires the following compounds: corn oil, methoxychlor, perylene and sulfur. The system calibration will determine the time spent from the injection of the sample to the elution of PAHs by using perylene as indicator. The fraction or fractions containing the PAHs can be selected by programming the fractions collector.

NOTE Other SEC columns can be used provided that the SEC system, after calibration, is able to include the PAHs in the selected fraction or fractions, with acceptable recovery rates and free of disturbing interferences.

5.14 HPLC apparatus, comprising the following:

5.14.1 Injection system, suitable for 100 µl injection volume.

5.14.2 Mobile phase pump (gradient), capable of pumping 1 ml/min pulse free.

5.14.3 Programmable fluorescence detector.

5.14.4 Computer based data processing system.

5.14.5 Analytical reverse-phase HPLC specific for PAHs, separating column, C18, base deactivated octadecyl silane (ODS) (recommended 4,6 mm x 250 mm column with 5 µm particle size), and a suitable corresponding reverse phase guard column.

5.14.6 Degasser, (optional).

5.14.7 Column oven, capable to operate at 28 °C ± 1 °C.

5.14.8 UV detector, capable to provide λ = 223 nm.

5.15 PTFE filters, 0,20 µm or 0,45 µm.

5.16 Glass tubes, of 10 ml capacity, suitable for the evaporator (5.4) and the fraction collector (5.13.5).

5.17 Glass tubes, of 10 ml capacity, capped.

5.18 Grinding mill.

6 Procedure

6.1 Sample preparation

In general, only the edible parts of foodstuffs will be analysed. The edible parts of shellfish shall be thoroughly washed with water and dried by slightly pressing with filter papers. The edible portions to be analysed shall be ground and well mixed. They can be stored in closed glass or aluminium containers under frozen conditions up to the moment of their analysis.

6.2 Extraction

Weigh, to the nearest 0,1 g, a 25 g test portion of prepared sample into a 250 ml-round bottomed glass tube, (5.3). Add 100 ml of dichloromethane (4.5), (V_1, see Clause 8). Blend for 3 min with a high speed blender (5.1).

Filter an aliquot of the lower organic layer through a prefolded paper filter. The filtered extract shall be clear, otherwise repeat this step with some anhydrous sodium sulphate (4.16) in the filter. Filter an aliquot of the filtered extract through a 0,20 µm or 0,45 µm PTFE filter (5.15), using a 5 ml glass syringe (5.7) and purify by SEC.

In the case of samples of edible oils, the extraction step is replaced by just a dispersion of the sample in dichloromethane as follows: Pour a 5 ml portion of oil in a previously tared glass tube (5.17) and take note of the sample weight to the nearest 0,1 g. Add 5 ml of dichloromethane (4.5), cap the tube and mix once by inversion. After that, mix by stirring one min in vortex mixer (5.1) and filter an aliquot of the mixture through a 0,20 µm or 0,45 µm PTFE filter (5.15).

NOTE 1 The amounts of oil and solvent can be modified depending on the convenience, but maintaining the same rate of 1 + 1 (for example 2 ml oil + 2 ml dichloromethane).

A mixture of 1 part per volume of cyclohexane and 1 part per volume of ethyl acetate (4.15) can be used as an alternative extraction solvent to dichloromethane. If so, it shall also be used for the equilibration, elution and washing of the SEC system (see 6.3).

NOTE 2 A mixture of 1 part per volume of cyclohexane and 1 part per volume of ethyl acetate gives longer elution times in the SEC and lower final recoveries than dichloromethane, although they always fulfil the legal requirements of the EU (see [3]).

6.3 SEC cleanup

Equilibrate the whole SEC system (5.13) previously by passing dichloromethane at a speed of 5 ml/min during approximately 30 min. Inject 0,2 ml (V_2, see Clause 8) of the filtered extract into the SEC system. Elute the PAHs by passing dichloromethane at a flow rate of 5 ml/min. Collect the fraction or fractions corresponding to the elution time of the PAHs according to the elution times established during the calibration of the SEC system. PAHs are eluted typically from 920 s to 1 170 s under the described conditions.

After the elution of the PAHs, equilibrate the SEC system again by passing dichloromethane during no less than 10 min before the next injection.

Evaporate the collected fraction or fractions to dryness in an evaporator under a gentle stream of nitrogen (for example, at 34 kPa (= 5 psi) and 27 °C to 28 °C), and redissolve in 1 ml (V_3, see Clause 8) of acetonitrile (4.6). If the HPLC is not performed immediately, store the final extract in a refrigerator.

7 HPLC analysis

7.1 HPLC operation conditions

When the column specified in (5.14.5) and the mobile phases A and B specified in 4.11 and 4.12 were used the setting as given in Table 1 were found to be appropriate:

Table 1 — Gradient conditions

Time min	**Flowrate** ml/min	**Mobile phase A** **%**	**Mobile phase B** **%**
0	1	50	50
3	1	50	50
30	1	0	100
40	1	0	100
43	2	0	100
70	2	0	100
85	1	50	50

NOTE Helium can be pumped or bubbled into the reservoirs of both mobile phases A and B for degassing. A degasser can be optionally used. Some commercial HPLC pumps have a degassing system incorporated.

The fluorescence wavelengths programme as given in Table 2 has been found to be appropriate for the detection of both the PAH4 (BaA, CHR, BbF and BaP) and the 15 EU priority fluorescent PAHs. If the aim of the analysis is just the determination of the PAH4, the gradient conditions (see Table 1) can be turned to initial conditions after BaP elution (at 32,4 min approximately) in order to shorten the run time: 50 % mobile phase A and 50 % mobile phase B. Consequently, the excitation and emission wavelengths (see Table 2) also can be turned to 230 nm (excitation) and 357 nm (emission).

Table 2 — Fluorescent wavelength programme

Time min	**Excitation wavelength** nm	**Emission wavelength** nm	**Gain**	**PAH**
0	230	357	1	BcL
23,80	280	410	10	BaA
25,30	270	385	10	CHR, 5MC
27,60	300	500	10	BjF, BbF
30,20	290	430	10	BkF, BaP, DIP, DhA, BgP
36,80	302	500	10	IcP
38,40	302	400	10	DeP
46,00	294	436	10	DiP
56,00	309	456	10	DhP

Detection of CPP can be performed by setting the wavelength of the UV detector at 223 nm.

7.2 Preparation of calibration solutions for HPLC

Prepare at least five HPLC calibration solutions by pipetting the volumes of the PAH4 or 15+1 PAH working standard solutions (4.20 or 4.22) or the calibration solutions of the appropriate concentration, as listed in Table 3, separately, into a set of flasks of 5 ml (5.9). To each flask, add the appropriate amount of acetonitrile for completing 5 ml. As an indication, these solutions cover a range from approximately 0,06 µg/kg to 100 µg/kg for each PAH under the conditions of this protocol for the analysis of edible oils, and from 0,12 µg/kg to 200 µg/kg in the case of 25 g sample weight for other matrices. Standards solutions 3 to 11 in Table 3 are obtained by diluting other standard solutions and not the working standard solutions. The analyst can choose the suitable calibration solutions for the most appropriate range to any given particular needs.

The solutions should be stored in capped vials under refrigeration conditions. Peak areas corresponding to a given PAH of the same calibration solution injected at regular intervals, should be within ± 3 %.

Table 3 — Preparation of HPLC calibration solutions

HPLC calibration solution	Standard solution		Final individual PAH concentration in calibration solution	
	Concentration	Volume		
	ng/ml	µl	ng/ml	m_a: ng (in 100 µl injection volume)
1	50,000	1 000	10,000	1,000 0
2	50,000	500	5,000	0,500 0
3	10,000	1 000	2,000	0,200 0
4	10,000	500	1,000	0,100 0
5	5,000	400	0,400	0,040 0
6	5,000	200	0,200	0,020 0
7	1,000	500	0,100	0,010 0
8	0,400	500	0,040	0,004 0
9	0,200	500	0,020	0,002 0
10	0,100	500	0,010	0,001 0
11	0,100	300	0,006	0,000 6

NOTE In case that the content of any individual PAH in the sample is outside of the calibration range, an appropriate calibration curve can be prepared. Alternatively, the injection solution for HPLC analysis can be diluted to a PAH content appropriate for the established calibration curve.

7.3 Calibration curve

Prepare a calibration curve by injecting 100 µl of five different PAHs calibration solutions (see 7.2). Plot the peak area against the mass of injected PAHs and check the curve for linearity.

7.4 Determination of PAHs in test solutions

Inject 100 µl aliquots of the test solutions into the chromatograph using the same conditions as for the preparation of the calibration curve. Identify each individual PAH peak of the test solution by comparing the retention time of the sample with that of the standard solutions.

8 Calculation

Determine from the calibration curve, the mass in ng of each PAH in the aliquot of test solution injected onto the HPLC column. Calculate the mass fraction, $w_{PAH,}$ of each PAH in micrograms per kilogram, using Formula (1):

$$w_{PAH} = m_a \times \frac{V_3 \times V_1}{V_4 \times V_2 \times m_s} \quad (1)$$

where

m_a is the mass of each PAH, in nanograms, in the aliquot of test solution injected and corresponding to the area of each PAH peak;

V_3 is the volume, in millilitres, of the final extract (usually V_3 = 1 ml);

V_1 is the volume, in millilitres, of the final extract in dichloromethane. In the case of oils, it is the sum of both volumes of dichloromethane and oil, respectively (usually V_1 = 100 ml);

V_4 is the volume, in millilitres, injected onto the HPLC column (usually V_4 = 0,1 ml);

V_2 is the volume, in millilitres, of the extract passed through the SEC system (usually V_2 = 0,2 ml);

m_s is the mass, in grams, of sample extracted (usually m_s = 25 g).

9 Recovery

The recovery shall be determined using certified reference materials (CRMs) or other methods approved by international guidelines, e.g. the use of surrogates such as spiking or internal standards (see [4] and [5]).

For analysis of the four PAHs in accordance with regulation (EU) No 836/2011 (see [3]), the recovery shall give values between 50 % and 120 %.

NOTE As an indication, the following CRMs are available to date: BCR®-459[1)] coconut oil PAHs blank, available from the Institute of Reference Materials and Measurement (IRMM), Geel, Belgium (http://irmm.jrc.ec.europa.eu/) and SRM®-1974b[1)] Organics in Mussel Tissue – Mytilus edulis, available from the National Institute for Standards and Technology (NIST), US Department of Commerce. (http://www.nist.gov/index.html).

10 Test report

The test report shall contain the following data:

a) all information necessary for the identification of the sample (kind of sample, origin and designation of the sample);

b) a reference to this Technical Specification;

c) the date and type of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

1) BCR®-459 and SRM®-1974b are examples of suitable products available commercially. This information is given for the convenience of the users of this Technical Specification and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they provide similar results.

f) the test results and the units in which they have been expressed;

g) operations not specified in the method or regarded as optional, which might have affected the results.

h) any other additional information required in the current applicable EU legislation

11 Precision data

11.1 General

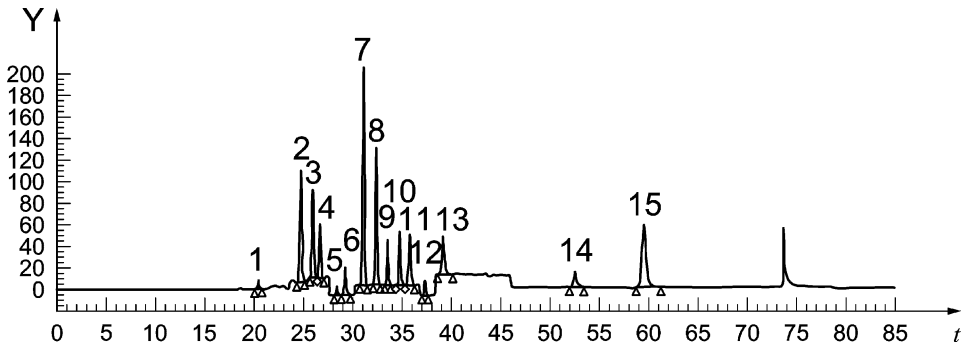
Details of the precision data obtained during the in-house validation study in terms of **within-laboratory** reproducibility are summarized in Annex B and Annex C.

11.2 Within-laboratory reproducibility

The absolute difference between two single test results found on identical test material under within-laboratory reproducibility conditions will exceed the within-laboratory reproducibility limit *R* in not more than 5 % of the cases.

Annex A
(informative)

Typical chromatograms



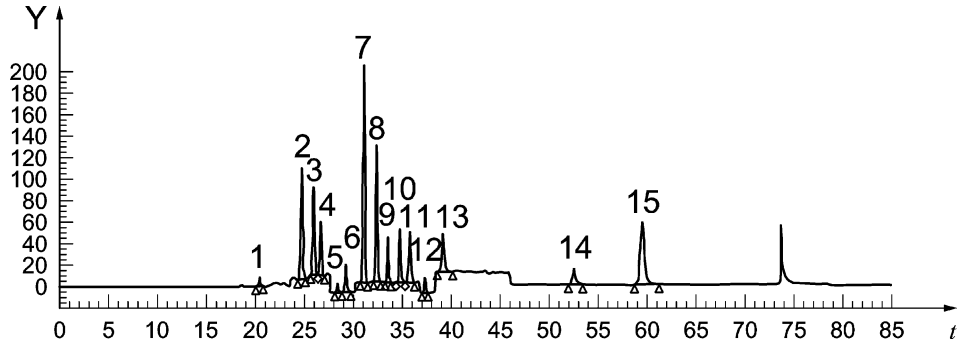
Key

t = time (min)

Y = fluorescence (mV)

Peak	1 = BcL	2 = BaA	3 = CHR	4 = 5MC	5 = BjF
identification	6 = BbF	7 = BkF	8 = BaP	9 = DlP	10 = DhA
	11 = BgP	12 = IcP	13 = DeP	14 = DiP	15 = DhP

Figure A.1 — Typical chromatogram of 15+1 PAHs from a sample of fresh mussels spiked at a level of 10 µg/kg, each (CPP cannot be detected by fluorescence at that level)



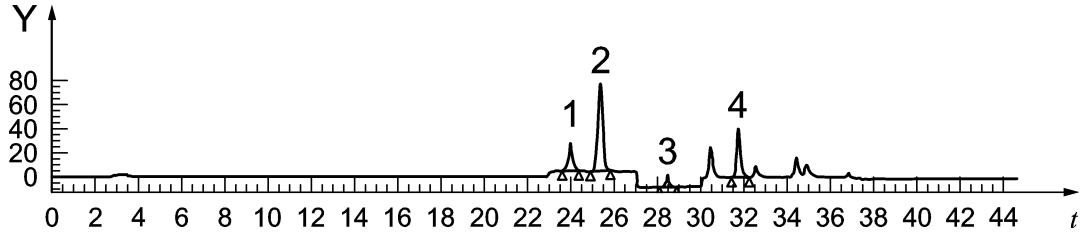
Key

t = time (min)

Y = fluorescence (mV)

Peak	1 = BcL	2 = BaA	3 = CHR	4 = 5MC	5 = BjF
identification	6 = BbF	7 = BkF	8 = BaP	9 = DlP	10 = DhA
	11 = BgP	12 = IcP	13 = DeP	14 = DiP	15 = DhP

Figure A.2 — Typical chromatogram of 15+1 PAHs from a sample of extra virgin olive oil spiked at a level of 2 µg/kg, each (CPP is cannot be detected by fluorescence at that level)



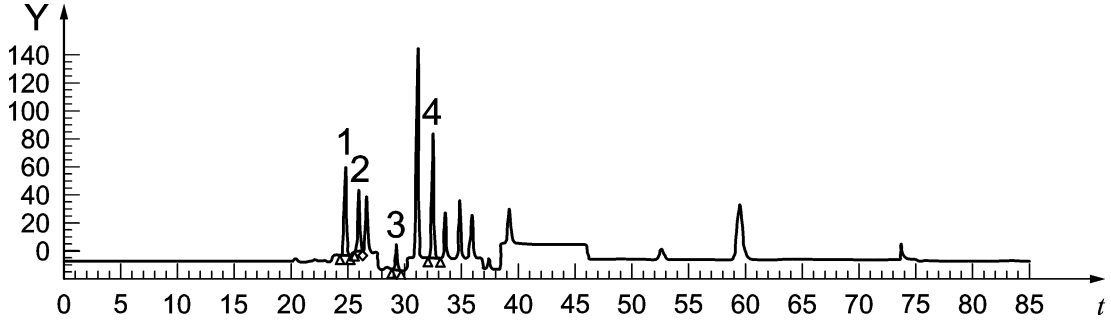
Key

t = time (min)

Y = fluorescence (mV)

Peak identification 1 = BaA 2 = CHR 3 = BbF 4 = BaP

Figure A.3 — Typical chromatogram of the PAH4 from a reference material of olive oil containing 2,06 µg/kg of BaP, 3,17 µg/kg of BaA, 2,40 µg/kg of BbF and 9,52 µg/kg of CHR, under the specific chromatographic conditions for the determination of the PAH4



Key

t = time (min)

Y = fluorescence (mV)

Peak identification 1 = BaA 2 = CHR 3 = BbF 4 = BaP

Figure A.4 — Typical chromatogram from a sample of processed cereal-based foods for young children spiked with the 15+1 PAH at the level of 10 µg/kg each, under the specific chromatographic conditions for the determination of the 15+1 PAHs. The chromatogram shows only the integration of the PAH4

Annex B
(informative)

In-house validation data for the PAH4 in different matrices

Details of the recovery data obtained during the in-house validation study are summarized in Annex B and Annex C. These recovery data were obtained by spiking samples of each type of food matrix. In some cases, samples used for spiking contained previously some amount of certain PAHs.

Table B.1 — In-house validation data for edible olive oil

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,39	2,36	22,00	0,42	2,10	22,83	1,24	3,13	24,22	0,55	2,52	25,06
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,10	0,17	1,42	0,03	0,32	2,15	0,09	0,21	2,31	0,08	0,17	0,86
Relative within-laboratory reproducibility, RSD_R, %	25,6	7,2	6,4	7,1	15,2	9,4	7,2	6,7	9,5	14,5	6,7	3,4
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,28	0,48	3,98	0,08	0,90	6,02	0,25	0,59	6,47	0,22	0,48	2,41
Recovery, %	84,8	99,4	100,9	95,9	87,8	97,3	105,6	98,5	99,5	91,4	100,1	105,8
Spiking level, µg/kg	0,41	2,34	23,34	0,40	2,35	23,41	0,41	2,37	23,53	0,41	2,37	23,53

Table B.2 — In-house validation data for fresh mussels

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,36	2,12	9,25	0,46	2,49	9,75	0,38	2,91	10,33	0,54	2,98	10,47
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,04	0,19	1,23	0,05	0,20	1,24	0,05	0,25	1,23	0,09	0,28	1,29
Relative within-laboratory reproducibility, RSD_R, %	11,1	9,0	13,3	10,9	8,0	12,7	13,2	8,6	11,9	16,7	9,4	12,3
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,11	0,53	3,44	0,14	0,56	3,47	0,14	0,70	3,44	0,25	0,78	3,61
Recovery, %	73,3	95,1	91,1	92,4	101,8	93,2	76,7	99,4	94,0	108,6	99,6	94,7
Spiking level, µg/kg	0,50	1,98	9,90	0,50	1,98	9,95	0,50	2,00	10,00	0,54	2,00	10,00

Table B.3 — In-house validation data for smoked fish

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,40	4,49	6,76	0,38	4,48	6,23	0,40	4,57	6,46	0,41	5,04	6,26
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,02	0,14	0,59	0,03	0,14	0,48	0,02	0,30	0,50	0,02	0,36	0,48
Relative within-laboratory reproducibility, RSD_R, %	5,0	3,1	8,7	7,9	3,1	7,7	5,0	6,6	7,7	4,9	7,1	7,7
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,06	0,39	1,65	0,08	0,39	1,34	0,06	0,84	1,40	0,06	1,01	1,34
Recovery, %	99,7	90,6	111,7	96,3	89,9	104,0	101,9	91,4	107,9	104,4	100,8	105,5
Spiking level, µg/kg	0,40	4,95	6,05	0,40	4,98	6,00	0,40	5,00	6,00	0,40	5,00	5,94

Table B.4 — In-house validation data for smoked meat products

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,38	1,83	4,70	0,37	1,74	4,78	0,39	1,76	4,67	0,39	1,94	4,66
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,02	0,06	0,26	0,03	0,09	0,40	0,02	0,12	0,31	0,02	0,09	0,15
Relative within-laboratory reproducibility, RSD_R, %	5,3	3,3	5,5	8,1	5,2	8,4	5,1	6,8	6,6	5,1	4,6	3,2
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,06	0,17	0,73	0,08	0,25	1,12	0,06	0,34	0,87	0,06	0,25	0,42
Recovery, %	94,6	92,4	95,5	93,0	87,6	96,6	98,5	88,0	92,7	98,5	97,2	93,5
Spiking level, µg/kg	0,40	1,98	4,93	0,40	1,99	4,95	0,40	2,00	4,95	0,40	2,00	4,98

Table B.5 — In-house validation data for processed cereal-based foods for young children

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,24	1,29	7,89	0,31	1,34	8,35	0,60	1,27	8,62	0,24	1,36	8,97
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,02	0,02	0,69	0,02	0,05	0,74	0,02	0,06	0,78	0,02	0	0,77
Relative within-laboratory reproducibility, RSD_R, %	8,3	1,6	8,7	6,5	3,7	8,9	3,3	4,7	9,0	8,3	0	8,6
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,06	0,06	1,93	0,06	0,14	2,07	0,06	0,17	2,18	0,06	0	2,16
Recovery, %	97,3	91,9	79,7	90,7	103,0	84,4	86,7	96,0	87,1	94,7	98,0	90,1
Spiking level, µg/kg	0,25	0,99	9,90	0,40	1,00	9,90	0,25	1,00	9,90	0,25	1,00	9,95

Table B.6 — In-house validation data for infant formulae

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,94	4,21	8,37	1,06	4,30	8,52	1,02	4,39	8,69	1,10	4,65	9,13
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,03	0,27	0,29	0,03	0,26	0,03	0,03	0,23	0,09	0,03	0,15	0,26
Relative within-laboratory reproducibility, RSD_R, %	3,2	6,4	3,5	2,8	6,0	0,4	2,9	5,2	1,0	2,7	3,2	2,8
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,08	0,76	0,81	0,08	0,73	0,08	0,08	0,64	0,25	0,08	0,42	0,73
Recovery, %	94,0	85,0	84,5	106,3	86,9	86,1	102,3	88,8	87,8	103,8	93,5	91,8
Spiking level, µg/kg	1,00	4,95	9,90	1,00	4,95	9,90	1,00	4,95	9,90	1,06	4,98	9,95

Table B.7 — In-house validation data for chocolate

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,48	1,85	4,53	0,42	1,78	4,53	0,60	2,13	4,68	0,57	2,13	4,81
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,04	0,30	0,21	0,01	0,24	0,21	0,03	0,32	0,20	0,02	0,27	0,21
Relative within-laboratory reproducibility, RSD_R, %	8,3	16,2	4,6	2,4	13,5	4,6	5,0	15,0	4,2	3,5	12,7	4,4
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,11	0,84	0,59	0,03	0,67	0,59	0,08	0,90	0,56	0,06	0,76	0,59
Recovery, %	91,1	87,1	87,0	87,6	82,8	91,7	95,6	91,8	89,2	98,2	96,1	96,7
Spiking level, µg/kg	0,40	1,98	4,95	0,40	1,98	4,95	0,40	1,98	4,95	0,40	1,99	4,98

Table B.8 — In-house validation data for food supplements (isoflavones)

	Benzo[*a*]pyrene			**Benz[*a*]anthracene**			**Chrysene**			**Benzo[*b*]fluoranthene**		
Mean value, $\bar{x}$, µg/kg	0,35	2,06	8,22	0,46	2,26	8,06	0,4	2,03	8,20	0,46	2,22	8,76
Within-laboratory reproducibility standard deviation SD_R, µg/kg	0,04	0,09	0,02	0,01	0,02	0,23	0,03	0,06	0,17	0,02	0,08	0,08
Relative within-laboratory reproducibility, RSD_R, %	11,4	4,4	0,2	2,2	0,9	2,9	7,5	3,0	2,1	4,3	3,6	0,9
Within-laboratory reproducibility limit R [$R = 2{,}8 \times SD_R$], µg/kg	0,11	0,25	0,06	0,03	0,06	0,64	0,08	0,17	0,48	0,06	0,22	0,22
Recovery, %	84,4	99,0	83,0	105,5	108,2	81,4	95,4	96,6	82,8	105,0	104,6	88,0
Spiking level, µg/kg	0,42	2,08	9,90	0,42	2,09	9,90	0,42	2,10	9,90	0,42	2,10	9,95

Annex C
(informative)

In-house performance data with a mixture of cyclohexane and ethyl acetate as alternative extraction solvent

Table C.1 — In-house performance data for olive oil

	BaA	CHR	BbF	BaP
Limit of quantification (LOQ), µg/kg	0,50	0,50	0,51	0,50
Recovery, %	78,0	86,0	93,5	85,3
Within-laboratory reproducibility, SD_R, %	3,9	12,0	4,8	2,1
Second level, µg/kg	1,99	1,99	2,00	1,99
Recovery, %	73,9	67,3	87,8	94,8
Within-laboratory reproducibility, SD_R, %	4,5	11,9	9,0	2,6
Third level, µg/kg	9,96	9,96	10,01	9,96
Recovery, %	62,4	65,1	74,7	73,9
Within-laboratory reproducibility, SD_R, %	1,2	0,9	5,9	0,6

Table C.2 — In-house performance data for fresh mussel

	BaA	CHR	BbF	BaP
Level, µg/kg	1,98	1,98	1,99	1,98
Recovery, %	88,0	82,7	92,0	103,2
Within-laboratory reproducibility, SD_R, %	9,5	15,9	16,2	9,8

Table C.3 — In-house performance data for smoked fish

	BaA	CHR	BbF	BaP
Limit of quantification (LOQ), µg/kg	0,50	0,50	0,50	0,50
Recovery, %	81,3	62,7	96,0	81,3
Within-laboratory reproducibility, SD_R, %	4,2	14,6	3,6	4,2
Second level, µg/kg	9,90	9,90	9,95	9,90
Recovery, %	70,0	76,8	84,1	85,1
Within-laboratory reproducibility, SD_R, %	4,7	6,9	4,8	3,6

Table C.4 — In-house performance data for infant formulae

	BaA	CHR	BbF	BaP
Limit of quantification (LOQ), µg/kg	0,40	0,40	0,41	0,40
Recovery, %	70,8	59,2	92,7	83,3
Within-laboratory reproducibility, SD_R, %	6,2	15,9	4,1	5,3
Second level, µg/kg	4,95	4,95	4,98	4,95
Recovery, %	60,2	65,3	73,5	74,4
Within-laboratory reproducibility, SD_R, %	8,4	7,7	4,5	9,8

Bibliography

[1] EN ISO 22959, *Animal and vegetable fats and oils - Determination of polycyclic aromatic hydrocarbons by on-line donor-acceptor complex chromatography and HPLC with fluorescence detection (ISO 22959)*

[2] EN ISO 15753, *Animal and vegetable fats and oils - Determination of polycyclic aromatic hydrocarbons (ISO 15753)*

[3] Commision Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

[4] Eurachem Guide, (1998), The fitness for Purpose of Analytical Methods – a Laboratory Guide to Method Validation and Related Topics

[5] THOMPSON M., ELLISON S.L.R., WOOD R. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis. IUPAC Technical Report. *Pure Appl. Chem.* 2002, **74** pp. 835–855

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