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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 gas chromatography mass spectrometry (GC-MS)

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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 gas chromatography mass spectrometry (GC-MS)

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 gazeuse couplée à la spectrométrie de masse (CG-SM)

Lebensmittelanalytik - Bestimmung von Benzo[a]pyren, Benz[a]anthracen, Chrysen und Benzo[b]fluoranthen in Lebensmitteln mit Gaschromatographie und Massenspektrometrie (GC-MS)

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EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 16619:2015) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.

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1 Scope

This European Standard specifies a method for the determination of 4 of the 16 EU priority polycyclic aromatic hydrocarbons (PAHs), identified as target PAHs. They are benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF) and chrysene (CHR). The method allows their quantification in the presence of the other 12 EU priority PAHs (benzo[*j*]fluoranthene (BjF), cyclopenta[*cd*]pyrene (CPP), benzo[*k*]fluoranthene (BkF), dibenz[*a,h*]anthracene (DhA), benzo[*c*]fluorene (BcL), 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 extruded wheat flour, smoked fish, dry infant formula, sausage meat, freeze-dried mussels, edible oil and wheat flour, by gas-chromatography mass-spectrometry (GC-MS). The extraction of PAHs from solid samples is performed by pressurized liquid extraction (PLE). Soxhlet extraction was applied by some participants in the method validation study by collaborative trial as alternative to PLE. The sample cleanup is performed by applying the following techniques in the reported sequence: size exclusion chromatography (SEC), and solid phase extraction (SPE).

This method complies with the performance characteristics specified in Commission Regulation (EU) No 836/2011 (see [1]). In particular the specifications for the limit of detection (LOD) and of the limit of quantification (LOQ) (0,30 µg/kg and 0,90 µg/kg respectively) were met.

The method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples, ranging from 0,5 µg/kg to 11,9 µg/kg. However, linearity of the instrument response was proven for the concentration range 0,5 µg/kg to 20 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, for more information see [2] and [3].

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 1042:1999, *Laboratory glassware - One-mark volumetric flasks (ISO 1042:1998)*

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

3 Principle

The sample is homogenized. A test portion is mixed with desiccant, sand and the stable isotope labelled internal standard solution. It is then extracted with *n*-hexane or cyclohexane by pressurized liquid extraction, or alternatively by Soxhlet extraction. If applicable, co-extracted water is separated from the organic phase of the extract. The organic extract is evaporated to a small volume, filtered and purified by SEC, using a mixture of ethyl acetate and cyclohexane as eluent.

After SEC, 200 µl of toluene are added as a keeper to the collected SEC fraction. The SEC fraction is evaporated to about 200 µl, and cleaned up by SPE on silica, using cyclohexane as eluent. The cleaned up sample extract is evaporated again to about 200 µl. Finally, an injection standard solution is added to the sample prior to measurement by GC-MS.

The injection is performed with a PTV, or split/splitless injection port. The chromatographic separation is obtained on a mid-polar capillary column with high selectivity for PAHs. The analytes are ionised by electron ionization (EI) at 70 eV. The target PAHs are recorded in Single Ion Monitoring (SIM) mode, and quantified by comparison with the stable isotope labelled analogues.

4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. All reagents and standard solutions shall be stored according to the specifications given by the supplier. The specifications given in this procedure for opened commercial solutions or for in-house prepared solutions aim to minimize solvent evaporation and to protect the analytes (PAHs) from degradation.

Standard solutions are preferably prepared gravimetrically. Depending on the handled amount of substance a micro-balance (6.4) and/or an analytical balance (6.5) are used for the preparation of solutions of both native and stable isotope labelled PAHs. All concentrations are expressed as mass per mass. If necessary, the concentrations expressed as mass per volume could be obtained applying the density equation (Formula (1)).

$$\rho = \frac{m}{v} \tag{1}$$

where

ρ density (in g/ml);

m measured mass of the substance (in g);

v volume of the solution (in ml).

The density of toluene at 20 °C is 0,8669 g/ml. Comprehensive information on the density of solvents at various temperatures is given in [4].

All solutions and substances are used at room temperature.

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 — Some precaution is needed when using plastics as polypropylene or PTFE because the analytes may be absorbed onto these materials.

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

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

4.4 Disodium sulfate, (Na_2SO_4), anhydrous, granular.

4.5 Poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide) granular, 90 µm to 850 µm particle size.

4.6 Sand, 50 mesh to 70 mesh particle size.

4.7 *n*-Hexane.

4.8 Acetone.

4.9 Cyclohexane.

4.10 Toluene.

4.11 Ethyl acetate.

4.12 SEC eluent

Mix 1 part per volume of cyclohexane (4.9) with 1 part per volume of ethyl acetate (4.11).

4.13 SPE column

For the solid phase extraction cleanup, a silica SPE column is used. Commercial cartridges of 500 mg – 4 ml or self-filled cartridges of the same size containing 500 mg activated silica are used. The surface area of the silica should be around 500 m^2/g.

NOTE Commercial SPE columns made of polypropylene were used in the method validation study by collaborative trial.

4.14 Reference material for quality control

A certified reference material, or any other suitable quality control material (e.g left over proficiency test material) may be applied for this purpose. The CITAC/Eurachem Guide to Quality in Analytical Chemistry may be consulted for guidance, see [5].

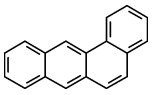
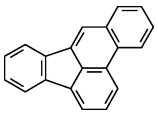
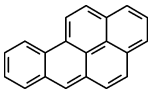
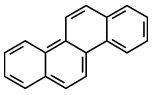
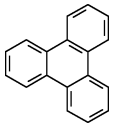
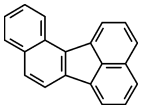
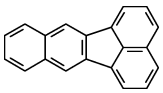
Analyse this material with every sample batch and use it to control the method performances along time (see 10.4).

4.15 Native reference substances - commercially available neat material or solutions of PAHs

The list of native substances analysed with this method is provided in Table 1. The target analytes are given in bold font. Commercially available, preferably certified, standard solutions are preferred due to the higher level of safety in handling.

Triphenylene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene are potentially interfering with the target analytes and are therefore used for evaluation of selectivity.

Table 1 — Names and structures of the native PAHs

Name[a]	CAS number	Structure	Name [a]	CAS number	Structure
Benz[*a*]anthracene (BaA)	56–55–3	 **Figure 1**	**Benzo[*b*]fluoranthene (BbF)**	205–99–2	 **Figure 2**
Benzo[*a*]pyrene (BaP)	50–32–8	 **Figure 3**	**Chrysene (CHR)**	218–01–9	 **Figure 4**
Triphenylene (TRP)	217–59–4	 **Figure 5**	Benzo[*j*]fluoranthene (BjF)	205–82–3	 **Figure 6**
Benzo[*k*]fluoranthene (BkF)	207–08–9	 **Figure 7**			

[a] The acronym is given in parenthesis, the target analytes are given in bold.

4.16 Stable isotope labelled reference standards (in the form of commercially available stable isotope labelled PAH solutions)

The stable isotope labelled analogues, applied for the quantification of the target PAHs are listed in Table 2. The commercial solutions used in the method validation study by collaborative trial contained the stable isotope labelled PAHs at a level of about 100 µg/kg in nonane.

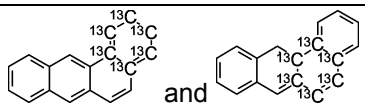
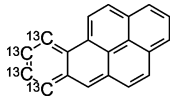
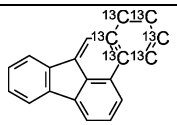
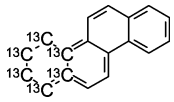
Preference is given to ^{13}C labelled analogues as their chemical properties best match those of the native analytes.

However, alternatively to ^{13}C labelled substances, deuterated analogues of the target analytes may be applied. The concentration levels of these solutions should be similar to the levels specified for the ^{13}C labelled PAH solutions.

NOTE 1 Highly deuterated PAHs are separated on the specified GC-column at least partially from their native analogues.

NOTE 2 Both forms of benz[*a*]anthracene-$^{13}C_6$, which are displayed in Table 2, are equally suitable for the purpose of this standard. The ^{13}C labelled reference material might be even supplied as a mixture, which was the case in the method validation study by collaborative trial.

Table 2 — Names and structures of ^{13}C labelled PAHs

Name	CAS number	Structure
Benz[*a*]anthracene-$^{13}C_6$	not available	 and **Figure 8**
Benzo[*a*]pyrene-$^{13}C_4$	not available	 **Figure 9**
Benzo[*b*]fluoranthene-$^{13}C_6$	not available	 **Figure 10**
Chrysene-$^{13}C_6$	not available	 **Figure 11**

The recovery of the method is calculated based on the stable isotope labelled standards (see 10.2). Their physical-chemical properties are considered equivalent to the native PAHs. Table 3 indicates the correspondence between each native PAH and the stable isotope labelled analogue applied for its quantification.

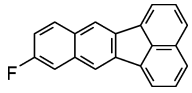
Table 3 — Correspondence stable isotope labelled PAHs and native PAHs

Stable isotope labelled PAHs	Native PAH [a]
Benz[*a*]anthracene $^{13}C_6$	Benz[*a*]anthracene (BaA)
Benzo[*a*]pyrene $^{13}C_4$	Benzo[*a*]pyrene (BaP)
Benzo[*b*]fluoranthene $^{13}C_6$	Benzo[*b*]fluoranthene (BbF)
Chrysene $^{13}C_6$	Chrysene (CHR)
[a] The acronym is given in parenthesis.	

4.17 9-fluorobenzo[*k*]fluoranthene (FBkF), as injection standard, neat or in form of a commercially available solution

9-fluorobenzo[*k*]fluoranthene (FBkF) is used as injection standard and added to the sample extract prior to injection into the GC-MS. Both neat material and commercially available solutions may be used. The concentration of a commercial solution is preferably 100 µg/kg in toluene.

Table 4 — Name and structure of the injection standard

Name [a]	CAS number	Structure
9-fluorobenzo[*k*]fluoranthene (FBkF)	113600–15–0	 **Figure 12**
[a] The acronym is given in parenthesis.		

5 Standard preparation

5.1 General

All standard solutions are preferably prepared gravimetrically. The tare masses of all recipients and the masses after each preparation step are recorded and used for calculation of the standard concentrations.

Volumetric preparation of standard solutions may be applied as well, provided that the used volumetric glassware complies with EN ISO 1042:1999.

Standard solutions may be prepared from neat materials, from commercial single substance solutions, or from commercial mixes. However, compatibility of the solvents of commercial solutions with toluene shall be taken into account.

Preferably, a commercial mixed PAH standard solution should be used for the preparation of intermediate standard solution (5.7) and calibration solutions (5.8). The concentration of this solution shall be in the same order of magnitude as the mixed PAH stock solution (5.4).

5.2 Injection standard solution

Prepare a solution of FBkF (4.17) in toluene (4.10) with a concentration of approximately 400 ng/g. The conversion of mass-per-mass units (ng/g) to mass-per-volume units (ng/ml) is done via the density equation (Formula (1)). For toluene a density value of 0,866 9 g/ml is applied.

This solution will be used for the spiking of the sample extract before measurement by GC-MS (see 7.9) to assess the recovery of stable isotope labelled PAHs (see 10.2).

Store this solution in the dark and at a temperature below 10 °C. A solution stored in this way is stable for at least six months. If longer stability is proven, the solution can still be applied.

5.3 PAH stock solutions

A solution in toluene (4.10) with a concentration in the range from 50 µg/g to 150 µg/g shall be prepared for each of the native PAHs listed in Table 1 in case neat reference materials are applied for the preparation of calibrants. These PAH stock solutions are prepared by weighing from 1 mg to 5 mg of each neat substance into glass weighing cylinders (6.1) using the microbalance (6.4). The weighing cylinder (6.1) is transferred with tweezers into a 100 ml amber glass volumetric flask (6.2). About 40 ml of toluene is added and weighed with an analytical balance (6.5). Other amber glass recipients may be applied, provided that solvent evaporation is minimized during standard preparation. Each flask shall be sonicated for a couple of minutes to achieve complete dissolution of the native PAHs in the solvent.

Once the solutions are homogeneous, they are transferred for storage into 40 ml amber glass vials (6.3). These solutions will be used for the preparation of mixed PAH stock solution (5.4) and, finally, of calibration solutions (see 5.8).

Store these solutions in the dark and at a temperature below 10 °C. A solution stored in this way is stable for at least 12 months. If longer stability is proven, the solution can still be applied.

5.4 Mixed PAH stock solutions

Prepare, from the PAH stock solutions (5.3), a solution in toluene (4.10) with a concentration of approximately 2 µg/g. For this purpose, both the PAH stock solutions (5.3) and the toluene (4.10) are weighed using an analytical balance (6.5).

This solution will be used for the preparation of the intermediate standard solutions (5.7) and, finally, of the calibration solutions (see 5.8).

Store this solution in the dark, at a temperature below 10 °C. A solution stored in this way was proven to be stable for at least 12 months. If longer stability is proven, the solution can still be applied.

5.5 Labelled PAH stock solution

Prepare with the individual solutions of stable isotope labelled PAHs (4.16) listed in Table 2, a solution in toluene (4.10) with a concentration of approximately 700 ng/g. Use an analytical balance (6.5) for this purpose.

This solution will be used for the preparation of the process solution (5.6).

Store this solution in the dark and at a temperature below 10 °C. A solution stored in this way is stable for at least 12 months. If longer stability is proven, the solution can still be applied.

5.6 Process solution

Prepare, from the Labelled PAH stock solution (5.5), a solution in toluene (4.10) with a concentration of approximately 150 ng/g. This concentration is obtained by adding 4,5 ml of the labelled PAH stock solution (5.5) to 16 ml of toluene (4.10). The exact amounts of the two components are determined gravimetrically with an analytical balance (6.5).

This solution will be used for spiking of the test portion (see 7.2).

Store this solution in the dark and at a temperature below 10 °C. A solution stored in this way is stable for at least six months. If longer stability is proven, the solution can still be applied.

5.7 Intermediate solutions

Prepare the intermediate solutions which will be used for calibration from the mixed PAH stock solution (5.4) by dilution in toluene (4.10).

These solutions will be used for the preparation of the calibration solutions (see 5.8).

The concentrations of PAHs in these solution shall be approximately two times the concentrations of PAHs in the calibration solutions (see 5.8), hence in the range from 10 ng/g to 250 ng/g. The required amounts of mixed PAH stock solution (5.4) are listed in Table 5. They are pipetted into a 100 ml amber volumetric flask (6.2) and made up to volume with toluene (4.10). The standard concentrations are calculated from gravimetrical data, which are recorded at each preparation step. Use an analytical balance (6.5) for weighing.

Table 5 — Nominal volumes of the mixed PAH stock solution (5.4) to be pipetted in order to prepare 100 ml of each of the listed concentration levels of the intermediate solutions

Intermediate solution	Nominal volume of mixed PAH stock solution (5.4) ml	Nominal PAH [a] concentration in the intermediate solution ng/g [b]
IS 1	0,5	10
IS 2	1,0	20
IS 3	2,5	50
IS 4	4,0	80
IS 5	5,5	110
IS 6	7,0	140
IS 7	8,5	170
IS 8	10,0	200
IS 9	12,5	250

[a] The concentration level refers to the individual target PAHs.

[b] The given concentrations are indicative. The real concentrations shall be determined from gravimetrical data.

Store these solutions in the dark and at a temperature below 10 °C. A solution stored in this way is stable for at least 12 months. If longer stability is proven, the solution can still be applied.

5.8 Calibration solutions

Prepare the calibration solutions from the intermediate solutions (5.7), the process solution (5.6), and the injection standard solution (5.2). Record the tare weight of the applied GC autosampler vials. Pipette directly in the GC autosampler vials (6.21.4), approximately 500 µl of the intermediate solutions (IS 1 to IS 9) (5.7), approximately 400 µl of the process solution (5.6), and approximately 100 µl of the injection standard solution (5.2). Weigh all solutions with an analytical balance (6.5). Calculate the concentrations from the gravimetrical data. The concentration levels of calibration solutions are summarized in Table 6.

Table 6 — Indicative compositions of the PAH calibration solutions

Calibration solution	PAH[a] concentration in the calibration solution ng/g [b]	Corresponding to PAH [a] concentration in the sample µg/kg	Stable isotope labelled PAH concentration in the calibration solution ng/g [b]	Concentration of FBkF in the calibration solution ng/g[b]
CS 1	5	0,5	60,0	40,0
CS 2	10	1,0	60,0	40,0
CS 3	25	2,5	60,0	40,0
CS 4	40	4,0	60,0	40,0
CS 5	55	5,5	60,0	40,0
CS 6	70	7,0	60,0	40,0
CS 7	85	8,5	60,0	40,0
CS 8	100	10,0	60,0	40,0
CS 9	125	12,5	60,0	40,0

[a] The concentration level refers to the 4 target PAHs.

[b] The final concentration shall be adjusted accordingly with the weighing and the exact concentration of the PAH in the mixed PAH stock solution (5.4).

5.9 Standard solution for assessment of chromatographic selectivity

Prepare a standard solution in toluene containing all PAHs listed in Table 1 (including triphenylene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene) at a concentration from 10 ng/g to 100 ng/g. This solution is used only for the characterization of the chromatographic separation (see 10.3).

6 Apparatus

WARNING — All glassware shall be meticulously cleaned (except disposable glassware). The glassware is first thoroughly washed with laboratory detergent and hot water. All glassware used for the preparation and storage of standards (e.g. glass weighing cylinders (6.1**) and amber volumetric flasks (**6.2**)) is rinsed before use with toluene (**4.10**) and dried in the fume hood under ambient conditions. Glassware used for other purposes (e.g. PLE solvent collection bottles (**6.8.6**)) is rinsed before use with cyclohexane and acetone (**4.8**) and dried either in the fume hood or in a drying cabinet.**

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

6.1 Glass cylinders, for weighing of neat PAHs, approximately of 1 ml volume, preferably made from amber glass.

Such vials are not commercialised for this particular purpose. However, several suppliers offer neckless, flat bottom glass vials with an outer diameter of 8 mm and a length of about 30 mm to 40 mm, which are suitable for the weighing of small amounts of solid substance.

6.2 Amber glass volumetric flasks, of various volumes (5 ml to 100 ml), according to EN ISO 1042:1999.

6.3 Amber glass vials, 40 ml, with PTFE layered screw caps.

6.4 Micro-balance, accuracy to the nearest 0,000 001 g.

6.5 Analytical balance, accuracy to the nearest 0,000 01 g.

6.6 Laboratory balance, accuracy to the nearest 0,01 g.

6.7 Porcelain mortar and pestle, capacity of the mortar shall be at least 200 ml.

6.8 Pressurized liquid extraction (PLE) apparatus[1)] comprising the following:

6.8.1 PLE cells, of 33 ml volume.

6.8.2 Cellulose filters, 30 mm diameter.

6.8.3 Sample carousel.

6.8.4 Degasser.

6.8.5 Extraction chamber.

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1) Dionex ASE® 300 is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products. Equivalent products may be used if they can be shown to lead to the same results.

6.8.6 Solvent collection bottles, compatible with the PLE apparatus, capable of collecting about 50 ml extract, with light protection.

6.8.7 Pressure control device, for the supply and release of the pressurizing gas (4.3) in the extraction cell.

6.8.8 Temperature control device.

6.8.9 Instrument control and data processing system.

6.9 Soxhlet apparatus (alternatively to PLE apparatus)

The apparatus comprises a heating mantle, a round bottom flask of 250 ml, a Soxhlet extractor of 85 ml capacity, a suitable cellulose thimble, a suitable condenser, and a cryostat or other cooling device.

6.10 Evaporation apparatus

An evaporator capable of evaporation under controlled temperature and vacuum condition, or an alternative concentration workstation shall be used for the evaporation of extracts. The evaporation apparatus shall be equipped with, either round bottom flasks or glass tubes, of appropriate volumes: approximately 250 ml for the evaporation of PLE extracts (approximately 100 ml), and 100 ml for evaporation of the collected eluent of SEC (approximately 50 ml).

6.11 Glass Pasteur capillary pipettes, 230 mm length.

6.12 Glass test tubes, 10 ml capacity.

6.13 Glass syringe, luer tip, 10 ml capacity.

6.14 Polytetrafluoroethylene (PTFE) membrane filter, diameter of 25 mm and 5 µm pore size.

6.15 Size Exclusion Chromatography (SEC) apparatus, comprising the following:

6.15.1 Liquid pump, supplying a flow rate of 4,0 ml/min.

6.15.2 Evaporating device (optional).

6.15.3 Sample carousel, for vials of 10 ml capacity (optional).

6.15.4 Amber sample collection vials, about 5 ml capacity.

6.15.5 Manual or automated injection system, capable of injecting 5 ml.

6.15.6 SEC column [2)]**, with the following characteristics:** 50 g of styrene-divenylbenzene polymer with 3 % crosslinkage, bead size 40 µm to 80 µm, in 25 mm x 500 mm glass column, preconditioned in a mixture of 1 part per volume of cyclohexane and 1 part per volume of ethyl acetate.

The SEC column shall be kept following the supplier specifications.

6.15.7 Solvent collection bottles.

6.15.8 Instrument control system, e.g. computer based.

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2) Bio-beads® S-X3 is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products. Equivalent products may be used if they can be shown to lead to the same results.

6.15.9 Autosampler vials for SEC apparatus.

6.16 Sample concentration apparatus, capable of evaporating small volumes under a stream of nitrogen (4.3) at controlled temperature.

6.17 Extraction manifold for solid phase extraction.

6.18 Disposable syringe barrels (optional), to be used as solvent reservoirs during SPE cleanup, 20 ml capacity, luer locks and attachments to fit onto the SPE cartridge (4.13).

6.19 Hand pump or similar device, to enhance flow through the SPE cartridge by a small overpressure.

6.20 Microlitre syringe(s) or calibrated microlitre pipette(s), with 25 µl to 500 µl capacity.

6.21 Gas-chromatography – mass spectrometry (GC-MS) apparatus, comprising the following:

6.21.1 Injection system, programmed temperature vaporising (PTV) injector, suitable for temperatures up to 400 °C.

Alternatively, a split/splitless injector, suitable for temperatures up to 350 °C, may be applied.

NOTE PTV injection allows injecting larger extract volumes compared to split/splitless injection. This provides larger signal intensities and might be advantageous with regard to LOD. However, split/splitless injection was proven in the method validation study by collaborative trial suitable for the scope of this standard.

6.21.2 GC oven, suitable for temperatures up to 325 °C and capable of temperature programming.

6.21.3 Sample carousel.

6.21.4 Amber sample vials for the sample carousel (6.21.3), with a capacity of about 2 ml.

6.21.5 GC capillary column, PAH selective capillary column[3)] length of 15 m, internal diameter of 0,15 mm, df = 0,10 µm (ß = 375), or any column with comparable separation characteristics.

This column, applied with the injection and oven conditions as described in 8.1, shall ensure at least the following resolutions:

BbF/BkF $R_s \geq 0,8$

BkF/BjF $R_s \geq 0,4$

CHR/TRP $R_s \geq 0,6$

6.21.6 An interface to the mass spectrometer, with a temperature control device, suitable for temperatures up to 350 °C (see 8.1.6).

6.21.7 A mass spectrometer, with the following characteristics:

— electron Ionization source;

— Ionization energy of 70 eV;

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3) Select PAH is an example of a commercially available capillary column that allows the separation of chrysene from its potential interference triphenylene (TRP). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

— mass resolution: at least 1 u;

— temperature control devices for the ion source (300 °C) and the GC-MS interface (325 °C). Optionally a temperature control device for the quadrupole (150 °C);

— tuning stability of at least 48 h (allowing for the analysis of a sequence of samples or standards);

— response linearity range of at least two orders of magnitude.

6.21.8 Computer based instrument control system, capable of programming gas chromatographic and mass spectrometric acquisition depending of run time.

6.21.9 Data processing system, computer based.

7 Procedure

7.1 Sample treatment

As a general precaution, all of the sample material received by the laboratory shall be used for obtaining a representative and homogenous test sample without introducing secondary contamination.

7.2 Test portion preparation

To obtain the test portion, weigh 5,0 g ± 0,1 g of the homogenized test sample into an aluminium weighing boat or equivalent tool, taking into account that plastic or PTFE materials should be avoided to reduce the risk of altering the test sample.

Transfer the test portion to the mortar (6.7), and add 5,0 g ± 0,2 g of polyacrylic acid (4.5) and 15,0 g ± 0,2 g of sand (4.6). Mix thoroughly until the sample is finely ground and visually homogeneous. Weigh the sand and the polyacrylic acid using a laboratory balance (6.6).

Add with a microlitre syringe, or calibrated microlitre pipette (6.20) 200 µl of process solution (5.6), homogenize and transfer the sample mixture to the PLE extraction cell. The nominal concentration of the stable isotope labelled standard substances in the test sample is about 5,2 µg/kg each.

The sample weight may be lowered if the analyte content exceeds the working range of this method.

7.3 Sample extraction by PLE and preparation for SEC

Place the cellulose filter (6.8.2) into the extraction cell. Then transfer the sample mixture spiked with the stable isotope labelled PAHs (5.6) into the extraction cell of the PLE apparatus (6.8.1). Add about 5 g of anhydrous Na_2SO_4 (4.4) into each collection bottle before starting extraction to bind the water potentially extracted with PLE. The small empty volume remaining in the extraction cell can be filled optionally with sand (4.6).

The extraction takes place under following conditions:

Pressure: 10 342 kPa (1 500 psi)

Temperature: 100 °C

Pre-heat time: 0 min

Heat time: 5 min

Static time: 10 min

Flush volume: 60 %

Purge time: 180 s

Static cycles: 2

Solvent: *n*-hexane 100 % (4.7) (alternatively cyclohexane 100 % (4.9)).

After the extraction, the extract is decanted into the evaporation vessel and the remaining Na_2SO_4 is washed 2 times with 10 ml of *n*-hexane (cyclohexane) which is transferred by a Pasteur pipette to the evaporation vessel and combined with the extract. Use a Pasteur pipette (6.11) both to mix the washing solvent with the Na_2SO_4 (and to wash the collection bottle walls in case fat drops should be present), and to transfer the washing solvent in the evaporation vessel. Avoid the transfer of Na_2SO_4. The prepared extract is concentrated to small volume (approximately 0,5 ml) in the evaporation apparatus (6.10).

The concentrated extract is transferred to a test tube (6.12), the evaporation vessel is washed with in total approximately 4 ml of the SEC eluent (4.12), which are combined with the sample extract in the same test tube. The final volume is adjusted with SEC eluent to approximately 5 ml.

7.4 Soxhlet extract as alternative to PLE

Transfer the sample mixture, prepared as described in 7.2, into the thimble of the Soxhlet apparatus (6.9). Top the sample with glass wool to avoid sample losses during re-condensation and reflux of the extraction solvent. Place the thimble in the Soxhlet extraction chamber. Add 200 ml of *n*-hexane (4.7) in the 250 ml round bottom flask (6.9). Set the extraction conditions so to obtain approximately 6 extraction cycles per hour. The duration of the extraction should be at least 7 h.

At the end of the extraction, add about 5 g of anhydrous Na_2SO_4 (4.4) to the extract in the round bottom flask and mix with the extract to bind potentially extracted water. Pour the extract into the evaporation vessel of the evaporation apparatus (6.10). Wash Na_2SO_4 remaining in the collection bottle 2 times with 10 ml of *n*-hexane (alternatively cyclohexane) which is transferred with a Pasteur pipette to the evaporation vessel and combined with the extract. Use a Pasteur pipette (6.11) both to mix the washing solvent and the Na_2SO_4 (and to wash the collection bottle walls in case fat drops should be present), and to transfer the washing solvent into the evaporation vessel. Avoid the transfer of Na_2SO_4.

Evaporate the solvent in the evaporation apparatus (6.10) to less than 2 ml. Avoid evaporation to dryness, as this could lead to losses. Transfer the concentrated extract to a test tube (6.12). Wash the evaporation vessel with approximately 3 ml of the SEC eluent (4.12), which are combined with the sample extract in the same test tube. Adjust the final volume with SEC eluent to approximately 5 ml.

7.5 SEC cleanup

Filter the 5 ml of test sample, prepared as described in 7.2 and 7.3 or 7.4, through a PTFE filter (6.14) by means of a glass syringe (6.13) into the SEC autosampler vial (6.15.9).

The SEC takes place under the following conditions:

Flow rate: 4 ml/min;

Eluent: cyclohexane: ethyl acetate 1:1 (4.12).

The elution times of the collected PAH fraction shall be adjusted according to the elution characteristics of the applied SEC column. This requires the determination of the elution profile of PAHs, by e.g collecting fractions of the eluent at regular intervals and determining the PAH content of each fraction.

7.6 Concentration step after SEC cleanup

Evaporate the collected fraction at the end of the SEC cleanup at 40 °C to about 5 ml.

7.7 Sample preparation for SPE cleanup

Transfer the concentrated SEC extract to a test tube (6.12) and add 200 µl of toluene (4.10), which serves as a keeper in the following evaporation steps. Evaporate the extract to approximately 200 µl in the sample concentrator (6.16) at 40 °C by applying a gentle stream of nitrogen (4.3) (the surface of the liquid should not be broken into drops). Then add 800 µl of cyclohexane (4.9) to the remaining 200 µl.

7.8 SPE cleanup

All elution steps are usually performed by gravity, without the use of any vacuum or pressure device. Only in case of high flow resistance a low vacuum or over-pressure is applied to facilitate elution. In this case, either a pressure device (6.19) or a SPE manifold (6.17) with attached vacuum system can be used.

Place the SPE columns onto the SPE manifold (6.17) or another suitable holder. Condition the SPE column (4.13) with 2 ml of cyclohexane (4.9) and discard the eluent.

Load the approximately 1 000 µl of the sample extract, obtained as described in 7.7, on the SPE column and let it infiltrate into the sorbent. Discard the eluate.

Elute the PAHs with 10 ml of cyclohexane (4.9). The first 2 ml are used to rinse the test tube. They are then loaded on the SPE column. The following 8 ml are either loaded in portions of 2 ml each or, if a solvent reservoir (6.18) is applied, in one step onto the SPE column. Let the solvent elute by gravity (about 1 ml/min) and collect the whole eluate in a test tube (6.12) till the flow stops.

7.9 Preparation of the sample for GC-MS analysis

Evaporate the collected SPE fraction at 40 °C by means of the sample concentrator (6.16) to about 200 µl, and then transfer it using a microlitre syringe (6.20) or other suitable device into a 2 ml amber autosampler vial (6.21.4). Rinse the test tube by using the same microlitre syringe twice with 100 µl of toluene and add both portions to the sample in the 2 ml vial (6.21.4). Finally, add 100 µl of injection standard solution (5.2) with a clean microlitre syringe (6.20) or other suitable device to the sample in the autosampler vial.

This final extract in toluene contains usually very low amounts of residual fat from the food matrix (below 0,01 %).

8 GC-MS analysis

8.1 GC-MS operating conditions

8.1.1 General

Satisfactory separation of the four marker PAHs from non-target PAHs is obtained with the gas-chromatographic column (6.21.5) and the following settings. However, the given parameter might not be applicable with all types of instruments. Modification and optimization might be required for gaining satisfactory separation.

NOTE 1 Information on instrument parameter settings applied by the participants in the method validation study by collaborative trial can be retrieved from EUR 25016 EN, see [9].

NOTE 2 Modifications of the oven programme within squared brackets allow the separation of all 16 EU priority PAHs as specified in Commission Regulation (EC) No 1881/2006, see [6]. They elute approximately between 10 min and 45 min.

8.1.2 Injection conditions, for PTV injector

- Injection mode: solvent vent
- Injection volume: 3 µl
- Injection speed: 5 µl/s
- Pre injection delay: 500 ms
- Post injection delay: 500 ms
- Initial temperature: 55 °C
- Initial time: 1,0 min
- First ramp: 600 °C/min up to 400 °C, static time 15 min
- Second ramp: 15 °C/min up to 70 °C, static time 0 min
- Vent time: 0,50 min
- Vent flow: 100 ml/min
- Vent pressure: 50 kPa
- Purge flow: 30 ml/min
- Purge time: 3 min
- Gas type: Helium (4.2)

8.1.3 Injection conditions, for split-splitless injector

- Injection mode: splitless
- Injection volume: 1 µl
- Temperature: 300 °C
- Purge flow: 30 ml/min
- Purge time: 2 min
- Total flow: 34,1 ml/min
- Gas type: Helium (4.2)

8.1.4 Oven conditions

- Initial temperature: 70 °C
- Initial time: 1 min
- First ramp: 60 °C/min up to 180 °C, static time 0 min
- Second ramp: 4 °C/min up to 230 °C, static time 10 min
- Third ramp: 28 °C/min up to 280 °C, static time 5 min [10 min]
- Fourth ramp: 60 °C/min up to 340 °C, static time 5 min [14 °C/min up to 340 °C, static time 5 min]

8.1.5 Column conditions

- Carrier gas flow: 1 ml/min, constant flow
- Gas type: Helium (4.2)

8.1.6 Transfer line conditions

— Temperature: 325 °C

8.1.7 Mass spectrometer conditions

— MS source temperature: 300 °C
— MS Quadrupole temperature: 150 °C
— Solvent delay: 7 min
— Electron Ionization Energy: 70 eV

8.1.8 Mass spectrometer acquisition parameters and peak identification

The acquisition of the m/z signals is performed in SIM mode according to Table 7. For quantification of all PAHs the peak area of the quantifier ion is used. However the peak is only considered identified, when the qualifier ion is present too and the ratio between the peak areas of quantifier and qualifier ions is within the acceptable range (see Table 8). The selected quantifier and qualifier ions and their ratio at the concentration corresponding to CS 5 (see Table 6) are listed in Table 8.

A typical GC-MS chromatogram of the 4 target PAHs in the presence of the other 12 EU PAHs at the concentration level of 100 ng/ml is shown in Figure A.1, Annex A.

Table 7 — Acquisition programme in SIM

Group number	**Initial time** min	**Dwell time** ms	**Ions** m/z
Group 1	15	40	114, 116 [a]
	15	80	228, 234[a]
Group 2	24	40	126, 128[a], 129[a], 135
	24	80	252, 256[a], 258[a], 270

[a] m/z ratios valid for ^{13}C labelled PAHs. They shall be adjusted if stable isotope labelled PAHs other than those in Table 3 listed are used.

Table 8 — Indicative retention times and ions for identification and quantification of the 4 target PAHs in SIM

Name [a]	Indicative retention time min	Quantifier ion Q_1 [b] m/z	Quantifier ion Q_2 [b] m/z	Peak area ratio Q_1/Q_2	Peak area ratio – lower limit	Peak area ratio – upper limit
Benz[*a*]anthracene (BaA)	19,01	228	114	12	6	18
Benzo[*a*]pyrene (BaP)	29,35	252	126	20	10	29
Benzo[*b*]fluoranthene (BbF)	26,65	252	126	13	6	20
Chrysene (CHR)	19,55	228	114	12	6	18
9-fluorobenzo[*k*]fluoranthene	25,76	270	135	15	7	22
Benz[*a*]anthracene $^{13}C_6$	19,01	234	116	10	5	15
Benzo[*a*]pyrene $^{13}C_4$	29,35	256	128	13	10	16
Benzo[*b*]fluoranthene $^{13}C_6$	26,66	258	129	12	10	14
Chrysene $^{13}C_6$	19,55	234	116	11	9	13

[a] For native PAHs, the acronym is given in parenthesis.

[b] With reference to Commission Decision (EC) 2002/657, see [7], a tolerance of ± 10 % to ± 50 % in the value of the ratio is accepted, depending of the amount of the diagnostic ion in relation with the target ion (for this method qualifier ion and quantifier ion respectively). A substance eluting from the chromatographic column is identified as one of the target analytes only:

– if the relative retention time of the unknown substance and the respective isotope labelled internal standard is within ± 0,5 % of the relative retention time of the analyte and the isotope labelled internal standard in the calibration solution;

– if both Q_1 and Q_2 are detected;

– if the ratio of Q_1 peak area and Q_2 peak area lies within the lower and upper limits listed in Table 8. Those limits were calculated applying Commission Decision 2002/657/EC criteria.

8.2 Calibration graph

Inject the calibration solutions listed in Table 6 at the beginning of every sequence. All solutions shall be brought to room temperature before injection. The injection of the calibration solutions shall be performed from the lower to the higher concentration so to reduce the risk of cross-contamination.

The calibration graph is obtained by plotting the peak area ratio of the target PAH and the corresponding stable isotope labelled analogue against the concentration of the target PAH in the calibration solutions. The calibration function is defined for each analyte by linear regression, and can be described by Formula (2).

$$\frac{A_{PAH-C}}{A_{LPAH-C}} = a \times \frac{[PAH]_C}{[LPAH]_C} + b \qquad (2)$$

where

A_{PAH-C} is the peak area of the quantifier ion for the native compound in the calibration solution;

A_{LPAH-C} is the peak area of the quantifier ion for the stable isotope labelled PAH in the calibration solution;

a is the slope of the calibration graph;

b is the intercept of the calibration graph;

$[PAH]_C$ is the concentration of native compound in the calibration solutions, in ng/g;

$[LPAH]_C$ is the concentration of the stable isotope labelled PAH in the calibration solutions, in ng/g.

The calibration curve should not be forced through the origin.

8.3 Sample analysis

Each sequence encompasses besides calibration solutions and sample extracts a procedural blank and the extract of a suitable quality control (QC) sample (10.4).

NOTE A procedural blank is a blank sample made up of all reagents foreseen for the preparation of the test portion and processed in all respects as the sample. This kind of blank tests the purity of the reagents but also identifies other possible sources of contamination, like the glassware and the analytical instrument (for this European Standard the procedural blank consists of 5 g of polyacrylic acid (5.5), 15 g of sand (5.6) and 200 µl of process solution (5.6)).

Before starting the sequence evaluate the system suitability based on at least one solvent blank and calibration solution CS 5. Inject the calibration solution in the order of increasing concentration (CS 1 to CS 9). Additional solvent blanks may be added in the sequence whenever the analyst considers it necessary.

After the calibration solutions, inject sample extracts, procedural blank samples and QC samples (10.4) in random order. Inject the calibration solutions CS 1 and CS 5 at the end of the sequence, or after at least 10 sample injections.

A typical sequence of analysis could be:

a) Instrumental performance verification:

   1) Solvent blank (toluene);

   2) CS 5;

b) Calibration solutions:

   1) Solvent blank (toluene, optional);

   2) CS 1 to CS 9 in ascending order;

   3) Solvent blank (toluene, optional);

c) Sample analysis:

   1) Solvent blank (toluene, optional);

   2) Sample extracts, procedural blank samples, QC samples (in random order, max 10 injections);

   3) CS 1;

   4) CS 5;

   5) Repetition of steps c) 2) to c) 4) if required;

   6) Solvent blank (toluene, optional).

9 Calculation and reporting

9.1 Calculation

For the calculation of the PAHs content of the sample Formula (3) is applied, where "*a*" (the slope of the calibration curve) and "*b*" (the intercept of the calibration curve) were obtained from the instrument calibration as described in 8.2.

$$[PAH]_{SAMPLE} = \left(\frac{\left(\frac{A_{PAH-S}}{A_{LPAH-S}} - b \right)}{a} \right) \times \frac{[LPAH] \times 0{,}8669 \times V_{LPAH-S}}{W_{SAMPLE}} \quad (3)$$

where

$[PAH]_{SAMPLE}$ is the concentration of the native compound in the sample, in µg/kg;

A_{PAH-S} is the peak area of the quantifier ion for the native compound in the sample;

A_{LPAH-S} is the peak area of the quantifier ion for the stable isotope labelled standard in the sample;

a is the slope of the calibration curve;

b is the intercept of the calibration curve;

[*LPAH*] is the concentration of the stable isotope labelled PAH in the process solution (5.6), in ng/g;

0,8669 is the density of toluene at 20 °C, in g/ml;

V_{LPAH-S} is the volume of the process solution (5.6) added to the sample, in ml;

W_{SAMPLE} mass of the test portion (7.2), in g.

9.2 Reporting

Do not report analysis results if the quality control criteria were not met (see Clause 10). Report the analysis results to the nearest 0,10 µg/kg. Report all results together with the corresponding expanded measurement uncertainty for which coverage factor of two shall be applied. In case the analyte content is below the limit of detection (LOD) or the limit of quantification (LOQ) report the result as below LOD or below LOQ respectively, and provide the concentration corresponding to the LOD / LOQ of the method.

If the calculated analyte content exceeds the upper limit of the working range, re-analyse the sample with an adjusted, lower sample weight.

10 Quality control

10.1 General

If quality control criteria specified in 10.2, 10.3 and 10.4 are met, accept and report the analytical results for the native compounds. In case one or the other is not met, review the analytical process for identification of sources and repeat the analysis of the concerned sample.

10.2 Recovery

It shall be stressed that recovery is only monitored for evaluation of compliance with legislation. Recovery values are not used for correction of results. The recovery of the PAH content in the sample is determined based on the stable isotope labelled PAHs, whose analytical behaviour is considered almost identical to that of native PAHs.

The recovery is estimated from the ratio of the relative response factors (RRF) of the stable isotope labelled PAHs and the injection standard (FBkF), determined for the test sample and the calibration solutions. A reference RRF is obtained from the calibration solutions CS 1 and CS 5 and is calculated with Formula (4) for each of the 4 target PAHs.

$$RRF_m = \frac{1}{n} \times \sum_{1}^{i} \frac{A_{LPAH,i} \times [IS]_i}{A_{IS,i} \times [LPAH]_i} \quad (4)$$

where

RRF_m is the mean RRF of the stable isotope labelled PAH in the calibration solutions;

$A_{LPAH,i}$ is the peak area of the quantifier ion of the stable isotope labelled PAH in calibration solution *i*;

$[IS]_i$ concentration of the injection standard (FBkF) (4.17) in calibration solution *i*, in ng/g;

$A_{IS,i}$ is the peak area of the quantifier ion of the injection standard in calibration solution *i*;

$[LPAH]_i$ is the concentration of the stable isotope labelled PAH in the calibration solution *i*, in ng/g.

The recovery of the stable isotope labelled PAHs, which is calculated as the percent ratio between the amount of stable isotope labelled PAH found in the sample and the amount spiked into the sample at the beginning of sample preparation, is obtained applying Formula (5).

$$\%REC_L = \frac{A_{LPAH-S} \times [IS] \times V_{IS-S}}{RRF_m \times A_{IS-S} \times [LPAH] \times V_{LPAH-S}} \times 100 \quad (5)$$

where

$\%REC_L$ is the percentage recovery of the stable isotope labelled PAH in the sample;

$A_{LPAH\text{-}S}$ is the peak area of the quantifier ion of the stable isotope labelled PAH in the GC-MS sample extract;

[*IS*] is the concentration of injection standard (FBkF) (4.17) in the injection standard solution (see 5.2), in ng/g;

$V_{IS\text{-}S}$ is the volume of the injection standard solution added to the sample (see 7.9), in ml;

RRF_m is the mean value of the RRF measured for the stable isotope labelled PAH in the calibration solution (from Formula (4));

$A_{IS\text{-}S}$ is the peak area of the quantifier ion of the injection standard solution in the GC-MS sample extract;

[*LPAH*] is the concentration of the stable isotope labelled PAH in the process solution (5.6), in ng/g;

$V_{LPAH\text{-}S}$ is the volume of the process solution added to the sample prior to extraction, in ml.

Recovery values between 50 % and 120 % are considered acceptable (Commission Regulation (EU) No 836/2011, see [1]).

10.3 Chromatographic resolution

Special attention shall be paid to the resolution between different PAHs and the stability of the retention times. The resolution between benzo[*b*]fluoranthene and benzo[*k*]fluoranthene as well as the resolution between benzo[*k*]fluoranthene and benzo[*j*]fluoranthene shall be monitored. The resolution is checked with the standard solution specified in 5.9 for the peak pairs benzo[*b*]fluoranthene (BbF) / benzo[*k*]fluoranthene, (BkF) the peak pair benzo[*k*]fluoranthene (BkF) / benzo[*j*]fluoranthene (BjF), and the peak pair chrysene (CHR) / triphenylene (TRP). Example chromatograms are given in Annex A.

The target resolution values are:

— BbF/BkF $R_s \geq 0,8$;

— BkF/BjF $R_s \geq 0,4$;

— CHR/TRP $R_s \geq 0,6$.

If this resolution is not achieved, perform root-cause analysis and re-start the analytical sequence after corrective action is implemented. Evaluate chromatographic resolution whenever modifications of the chromatographic system could cause changes in resolution values, e.g. cutting the analytical column, installing a new column, etc.

10.4 Reference material for method performance check

To check method performance along time, analyse a reference material during the analytical sequence and plot the result in a quality control chart. A certified reference material, a proficiency test (PT) material, or a self-prepared laboratory reference material could be used for this purpose. Guidance on the set up and interpretation of quality control charts may be taken from relevant guidance documents, e.g. the IUPAC Harmonized Guidelines for Internal Quality Control in Analytical Chemistry, see [8].

10.5 Procedural blank

The analyte content of the procedural blank shall not exceed the level of 0,20 µg/kg per analyte. Calculate the analyte content of the procedural blank with the calibration function given in 8.2. In case of exceeding the level, the source of contamination shall be identified and contamination shall be remediated.

10.6 Integrity check of the standards

The mass of each standard preparation (storage vial plus content plus closure) should be recorded before storing it. Before using a standard solution the mass of the standard preparation should be determined. In comparing the actual mass with the mass recorded after preparation/previous use, potential losses of solvent can be determined. If the deviation between the actual mass and the mass before storage is larger than 0,5 % of the mass before storage, the actual concentration of the standard preparation has to be recalculated from the density equation.

11 Precision data

11.1 General

Details of the interlaboratory test of the precision of the method are summarized in Annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and/or matrices other than those given in Annex B.

11.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit *r* in not more than 5 % of the cases.

The values for benz[*a*]anthracene are:

$\bar{x}$ = 0,6 µg/kg	*r* = 0,1 µg/kg	(extruded wheat flour)
$\bar{x}$ = 3,5 µg/kg	*r* = 0,8 µg/kg	(smoked fish)
$\bar{x}$ = 1,3 µg/kg	*r* = 0,3 µg/kg	(dry infant formula A)
$\bar{x}$ = 5,0 µg/kg	*r* = 0,6 µg/kg	(dry infant formula B)
$\bar{x}$ = 2,8 µg/kg	*r* = 0,3 µg/kg	(sausage meat)
$\bar{x}$ = 2,7 µg/kg	*r* = 0,7 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 4,1 µg/kg	*r* = 1,0 µg/kg	(edible oil A)
$\bar{x}$ = 7,9 µg/kg	*r* = 2,6 µg/kg	(edible oil B)
$\bar{x}$ = 1,0 µg/kg	*r* = 0,2 µg/kg	(wheat flour)

The values for benzo[*a*]pyrene are:

$\bar{x}$ = 0,5 µg/kg	*r* = 0,2 µg/kg	(extruded wheat flour)
$\bar{x}$ = 9,2 µg/kg	*r* = 0,7 µg/kg	(smoked fish)
$\bar{x}$ = 0,6 µg/kg	*r* = 0,3 µg/kg	(dry infant formula A)
$\bar{x}$ = 4,8 µg/kg	*r* = 0,7 µg/kg	(dry infant formula B)
$\bar{x}$ = 2,2 µg/kg	*r* = 0,2 µg/kg	(sausage meat)
$\bar{x}$ = 0,9 µg/kg	*r* = 0,2 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 4,7 µg/kg	*r* = 0,7 µg/kg	(edible oil A)
$\bar{x}$ = 11,9 µg/kg	*r* = 0,7 µg/kg	(edible oil B)
$\bar{x}$ = 0,7 µg/kg	*r* = 0,2 µg/kg	(wheat flour)

The values for benzo[*b*]fluoranthene are:

$\bar{x}$ = 0,8 µg/kg	*r* = 0,1 µg/kg	(extruded wheat flour)
$\bar{x}$ = 4,7 µg/kg	*r* = 0,7 µg/kg	(smoked fish)
$\bar{x}$ = 2,7 µg/kg	*r* = 1,0 µg/kg	(dry infant formula A)
$\bar{x}$ = 4,2 µg/kg	*r* = 0,7 µg/kg	(dry infant formula B)
$\bar{x}$ = 2,2 µg/kg	*r* = 0,2 µg/kg	(sausage meat)
$\bar{x}$ = 4,4 µg/kg	*r* = 1,0 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 10,4 µg/kg	*r* = 0,7 µg/kg	(edible oil A)
$\bar{x}$ = 5,3 µg/kg	*r* = 0,6 µg/kg	(edible oil B)
$\bar{x}$ = 1,6 µg/kg	*r* = 0,8 µg/kg	(wheat flour)

The values for chrysene are:

$\bar{x}$ = 0,8 µg/kg	*r* = 0,1 µg/kg	(extruded wheat flour)
$\bar{x}$ = 5,4 µg/kg	*r* = 0,8 µg/kg	(smoked fish)
$\bar{x}$ = 0,9 µg/kg	*r* = 0,4 µg/kg	(dry infant formula A)
$\bar{x}$ = 3,3 µg/kg	*r* = 0,4 µg/kg	(dry infant formula B)
$\bar{x}$ = 3,1 µg/kg	*r* = 0,7 µg/kg	(sausage meat)
$\bar{x}$ = 4,7 µg/kg	*r* = 1,2 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 6,2 µg/kg	*r* = 0,5 µg/kg	(edible oil A)
$\bar{x}$ = 7,7 µg/kg	*r* = 2,0 µg/kg	(edible oil B)
$\bar{x}$ = 1,6 µg/kg	*r* = 0,6 µg/kg	(wheat flour)

11.3 Reproducibility

The absolute difference between two single test results found on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

The values for benz[*a*]anthracene are:

$\bar{x}$ = 0,6 µg/kg	*R* = 0,3 µg/kg	(extruded wheat flour)
$\bar{x}$ = 3,5 µg/kg	*R* = 1,3 µg/kg	(smoked fish)
$\bar{x}$ = 1,3 µg/kg	*R* = 0,6 µg/kg	(dry infant formula A)
$\bar{x}$ = 5,0 µg/kg	*R* = 1,2 µg/kg	(dry infant formula B)
$\bar{x}$ = 2,8 µg/kg	*R* = 1,0 µg/kg	(sausage meat)
$\bar{x}$ = 2,7 µg/kg	*R* = 2,6 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 4,1 µg/kg	*R* = 1,0 µg/kg	(edible oil A)
$\bar{x}$ = 7,9 µg/kg	*R* = 3,2 µg/kg	(edible oil B)
$\bar{x}$ = 1,0 µg/kg	*R* = 0,4 µg/kg	(wheat flour)

The values for benzo[*a*]pyrene are:

$\bar{x}$ = 0,5 µg/kg	*R* = 0,4 µg/kg	(extruded wheat flour)
$\bar{x}$ = 9,2 µg/kg	*R* = 2,4 µg/kg	(smoked fish)
$\bar{x}$ = 0,6 µg/kg	*R* = 0,5 µg/kg	(dry infant formula A)
$\bar{x}$ = 4,8 µg/kg	*R* = 1,4 µg/kg	(dry infant formula B)
$\bar{x}$ = 2,2 µg/kg	*R* = 0,7 µg/kg	(sausage meat)
$\bar{x}$ = 0,9 µg/kg	*R* = 1,3 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 4,7 µg/kg	*R* = 1,3 µg/kg	(edible oil A)
$\bar{x}$ = 11,9 µg/kg	*R* = 2,4 µg/kg	(edible oil B)
$\bar{x}$ = 0,7 µg/kg	*R* = 0,3 µg/kg	(wheat flour)

The values for benzo[*b*]fluoranthene are:

$\bar{x}$ = 0,8 µg/kg	*R* = 0,5 µg/kg	(extruded wheat flour)
$\bar{x}$ = 4,7 µg/kg	*R* = 1,4 µg/kg	(smoked fish)
$\bar{x}$ = 2,7 µg/kg	*R* = 1,4 µg/kg	(dry infant formula A)
$\bar{x}$ = 4,2 µg/kg	*R* = 1,4 µg/kg	(dry infant formula B)
$\bar{x}$ = 2,2 µg/kg	*R* = 0,8 µg/kg	(sausage meat)
$\bar{x}$ = 4,4 µg/kg	*R* = 2,0 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 10,4 µg/kg	*R* = 2,4 µg/kg	(edible oil A)
$\bar{x}$ = 5,3 µg/kg	*R* = 1,5 µg/kg	(edible oil B)
$\bar{x}$ = 1,6 µg/kg	*R* = 1,1 µg/kg	(wheat flour)

The values for chrysene are:

$\bar{x}$ = 0,8 µg/kg	*R* = 0,4 µg/kg	(extruded wheat flour)
$\bar{x}$ = 5,4 µg/kg	*R* = 1,6 µg/kg	(smoked fish)
$\bar{x}$ = 0,9 µg/kg	*R* = 0,8 µg/kg	(dry infant formula A)
$\bar{x}$ = 3,3 µg/kg	*R* = 0,9 µg/kg	(dry infant formula B)
$\bar{x}$ = 3,1 µg/kg	*R* = 1,3 µg/kg	(sausage meat)
$\bar{x}$ = 4,7 µg/kg	*R* = 4,6 µg/kg	(freeze-dried mussels)
$\bar{x}$ = 6,2 µg/kg	*R* = 1,9 µg/kg	(edible oil A)
$\bar{x}$ = 7,7 µg/kg	*R* = 3,0 µg/kg	(edible oil B)
$\bar{x}$ = 1,6 µg/kg	*R* = 1,3 µg/kg	(wheat flour)

12 Test report

The test report shall contain the following data:

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

b) a reference to this European Standard;

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

d) the date of receipt;

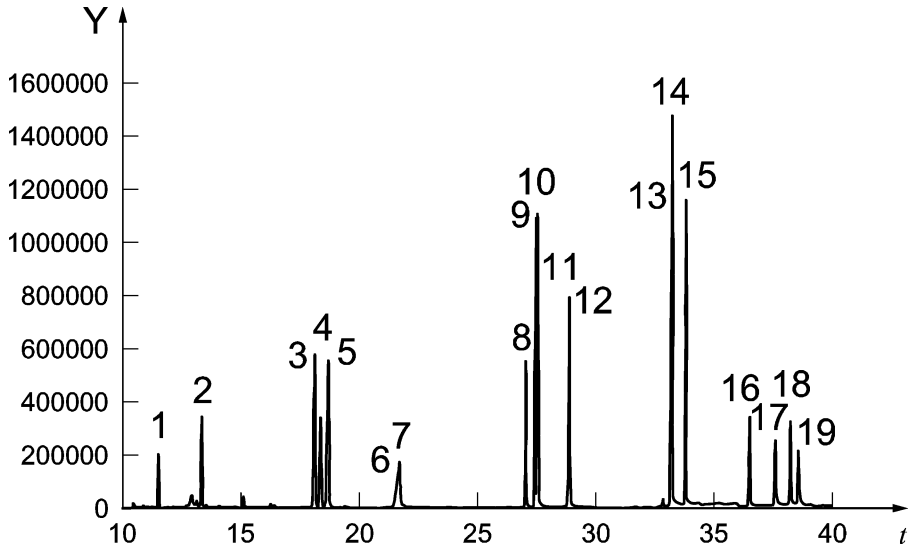
e) the date of test;

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

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

Annex A
(informative)

Typical chromatograms



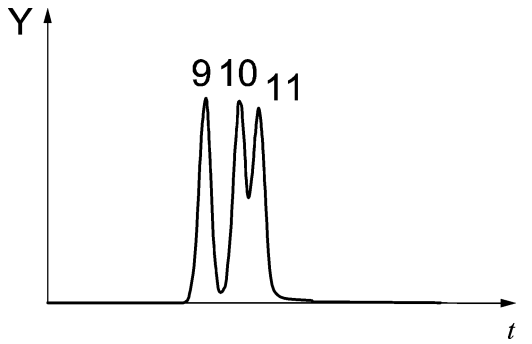
Key

t = time, in min

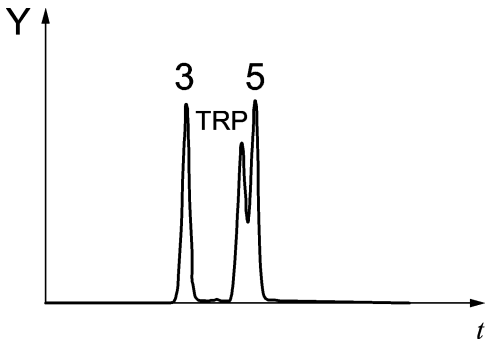
Y = abundance

The identity of the peaks is given in Table A.1 (TIC: total ion current).

Figure A.1 — Typical GC-MS chromatogram of 15+1 EU PAHs and stable isotope labelled PAHs obtained under the conditions described in Clause 8 and applying PTV injection



a) Separation of benzofluoranthene isomers (base peak ion chromatogram at m/z = 252)



b) Separation of triphenylene (TRP) from chrysene (5) (base peak ion chromatogram at m/z = 228)

Key

t = time, in min

Y = abundance

Figure A.2 — Extracts from chromatograms of PAH standard solutions in toluene

Table A.1 — Identification of chromatographic peaks

Name[a]	Peak number	Name [a]	Peak number	Name[a]	Peak number
Pyrene $^{13}C_3$	1	Benzo[*b*]fluoranthene (BbF)	9	Dibenz[*a,h*]anthracene $^{13}C_6$	14
Benzo[*c*]fluorene (BcL)	2	Benzo[*b*]fluoranthene $^{13}C_6$	9	Benzo[*ghi*]perylene (BgP)	15
Benz[*a*]anthracene (BaA)	3	Benzo[*k*]fluoranthene (BkF)	10	Benzo[*ghi*]perylene $^{13}C_{12}$	15
Benz[*a*]anthracene $^{13}C_6$	3	Benzo[*k*]fluoranthene $^{13}C_6$	10	Dibenzo[*a,l*]pyrene (DlP)	16
Cyclopenta[*cd*]pyrene (CPP)	4	Benzo[*j*]fluoranthene (BjF)	11	Dibenzo[*a,e*]pyrene (DeP)	17
Chrysene (CHR)	5	Benzo[*a*]pyrene (BaP)	12	Dibenzo[*a,e*]pyrene $^{13}C_6$	17
Chrysene $^{13}C_6$	5	Benzo[*a*]pyrene $^{13}C_4$	12	Dibenzo[*a,i*]pyrene (DiP)	18
5-Methylchrysene methyl D_3	6	Indeno[1,2,3-*cd*]pyrene (IcP)	13	Dibenzo[*a,i*]pyrene $^{13}C_{12}$	18
5-Methylchrysene (5MC)	7	Indeno[1,2,3-*cd*]pyrene $^{13}C_6$	13	Dibenzo[*a,h*]pyrene (DhP)	19
9-Fluorobenzo[*k*]fluoranthene	8	Dibenz[*a,h*]anthracene (DhA)	14		
[a] For native PAHs the acronym is given in parenthesis.					

Operating conditions for Figure A.1:

Column:	Select PAH, length of 15 m, internal diameter of 0,15 mm, df = 0,10 µm
Initial flow rate:	1 ml/min
Carrier gas:	Helium (4.2)
Column temperature:	See oven programme (8.1.4)
Injection volume:	3 µl (for programmed temperature vaporizer injection (PTV))
Detection:	m/z ions listed in Table 7 in the conditions reported in 8.1.7 and 8.1.8.

Annex B
(informative)

Precision data

The data given in Tables B.1, B.2, B.3 and B.4 were obtained in an interlaboratory study (see [9]), organized by the Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC-IRMM) in accordance with ISO 5725-2 [10] for collaborative study procedures to validate characteristics of a method of analysis.

Table B.1 — Statistical evaluation of interlaboratory study results for benz[*a*]anthracene in different food

Sample	Extruded wheat flour	Smoked fish	Dry infant formula A	Dry infant formula B	Sausage meat	Freeze-dried mussels	Edible oil A	Edible oil B	Wheat flour
Preparation of test material	naturally incurred	spiked	spiked	spiked	spiked	naturally incurred	spiked	spiked	naturally incurred
Year of interlaboratory test	2010	2010	2010	2010	2010	2010	2010	2010	2010
Number of laboratories	11	11	11	11	11	10	11	11	11
Number of laboratories considered as non-compliant	0	0	1	0	2	0	0	0	0
Number of laboratories retained after eliminating outliers	10	11	9	10	9	10	10	11	10
Number of outliers (laboratories)	1	0	1	1	0	0	1	0	1
Number of accepted results	10	11	9	10	9	10	10	11	10
Mean value, $\bar{x}$, µg/kg	0,6	3,5	1,3	5,0	2,8	2,7	4,1	7,9	1,0
Repeatability standard deviation s_r, µg/kg	0,04	0,3	0,1	0,2	0,1	0,2	0,3	0,9	0,1
Repeatability relative standard deviation, RSD_r, %	7	8	9	4	5	9	8	12	8
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	0,1	0,8	0,3	0,6	0,3	0,7	1,0	2,6	0,2
Reproducibility standard deviation s_R, µg/kg	0,1	0,5	0,2	0,4	0,4	0,9	0,4	1,1	0,1
Reproducibility relative standard deviation, RSD_R, %	20	13	16	9	13	35	9	14	14
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	0,3	1,3	0,6	1,2	1,0	2,6	1,0	3,2	0,4
Recovery[a], %	66	67	66	65	71	not calculated	73	74	66
HorRat value, according to [11]	0,92	0,59	0,73	0,40	0,60	1,57	0,40	0,66	0,66

[a] Recovery values are based on the recovery of stable isotope labelled PAHs.

Table B.2 — Statistical evaluation of interlaboratory study results for benzo[*a*]pyrene in different food

Sample	Extruded wheat flour	Smoked fish	Dry infant formula A	Dry infant formula B	Sausage meat	Freeze-dried mussels	Edible oil A	Edible oil B	Wheat flour
Preparation of test material	naturally incurred	spiked	spiked	spiked	spiked	naturally incurred	spiked	spiked	naturally incurred
Year of interlaboratory test	2010	2010	2010	2010	2010	2010	2010	2010	2010
Number of laboratories	11	11	11	11	11	10	11	11	11
Number of laboratories considered as non-compliant	0	0	2	0	2	1	0	0	0
Number of laboratories retained after eliminating outliers	9	9	8	10	9	8	10	9	9
Number of outliers (laboratories)	2	2	1	1	0	1	1	2	2
Number of accepted results	9	9	8	10	9	8	10	9	9
Mean value, $\bar{x}$, µg/kg	0,5	9,2	0,6	4,8	2,2	0,9	4,7	11,9	0,7
Repeatability standard deviation s_r, µg/kg	0,1	0,3	0,1	0,2	0,1	0,1	0,2	0,3	0,1
Repeatability relative standard deviation, RSD_r, %	12	3	15	5	4	8	5	2	13
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	0,2	0,7	0,3	0,7	0,2	0,2	0,7	0,7	0,2
Reproducibility standard deviation s_R, µg/kg	0,1	0,9	0,2	0,5	0,3	0,5	0,5	0,8	0,1
Reproducibility relative standard deviation, RSD_R, %	24	9	30	11	12	54	10	7	18
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	0,4	2,4	0,5	1,4	0,7	1,3	1,3	2,4	0,3
Recovery[a], %	79	78	72	75	76	not calculated	80	78	78
HorRat value, according to [11]	1,09	0,43	1,37	0,49	0,56	2,46	0,44	0,32	0,84

[a] Recovery values are based on the recovery of stable isotope labelled PAHs.

Table B.3 — Statistical evaluation of interlaboratory study results for benzo[*b*]fluoranthene in different food

Sample	Extruded wheat flour	Smoked fish	Dry infant formula A	Dry infant formula B	Sausage meat	Freeze-dried mussels	Edible oil A	Edible oil B	Wheat flour
Preparation of test material	naturally incurred	spiked	spiked	spiked	spiked	naturally incurred	spiked	spiked	naturally incurred
Year of interlaboratory test	2010	2010	2010	2010	2010	2010	2010	2010	2010
Number of laboratories	11	11	11	11	11	10	11	11	11
Number of laboratories considered as non-compliant	1	1	2	1	2	1	1	1	1
Number of laboratories retained after eliminating outliers	8	9	9	10	9	8	9	9	10
Number of outliers (laboratories)	2	1	0	0	0	1	1	1	0
Number of accepted results	8	9	9	10	9	8	9	9	10
Mean value, $\bar{x}$, µg/kg	0,8	4,7	2,7	4,2	2,2	4,4	10,4	5,3	1,6
Repeatability standard deviation s_r, µg/kg	0,04	0,3	0,4	0,2	0,1	0,4	0,2	0,2	0,3
Repeatability relative standard deviation, RSD_r, %	5	5	14	6	4	8	2	4	17
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	0,1	0,7	1,0	0,7	0,2	1,0	0,7	0,6	0,8
Reproducibility standard deviation s_R, µg/kg	0,2	0,5	0,5	0,5	0,3	0,7	0,9	0,5	0,4
Reproducibility relative standard deviation, RSD_R, %	22	10	18	12	13	16	8	10	25
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	0,5	1,4	1,4	1,4	0,8	2,0	2,4	1,5	1,1
Recovery[a], %	77	71	74	75	72	not calculated	79	79	74
HorRat value, according to [11]	0,99	0,47	0,81	0,53	0,60	0,75	0,38	0,45	1,15

[a] Recovery values are based on the recovery of stable isotope labelled PAHs.

Table B.4 — Statistical evaluation of interlaboratory study results for chrysene in different food

Sample	Extruded wheat flour	Smoked fish	Dry infant formula A	Dry infant formula B	Sausage meat	Freeze-dried mussels	Edible oil A	Edible oil B	Wheat flour
Preparation of test material	naturally incurred	spiked	spiked	spiked	spiked	naturally incurred	spiked	spiked	naturally incurred
Year of interlaboratory test	2010	2010	2010	2010	2010	2010	2010	2010	2010
Number of laboratories	11	11	11	11	11	10	11	11	11
Number of laboratories considered as non-compliant	0	0	1	0	2	0	0	0	0
Number of laboratories retained after eliminating outliers	9	10	10	9	9	10	9	10	10
Number of outliers (laboratories)	2	1	0	2	0	0	2	1	0
Number of accepted results	9	10	10	9	9	10	9	10	10
Mean value, $\bar{x}$, µg/kg	0,8	5,4	0,9	3,3	3,1	4,7	6,2	7,7	1,6
Repeatability standard deviation s_r, µg/kg	0,0	0,3	0,2	0,1	0,2	0,4	0,2	0,7	0,2
Repeatability relative standard deviation, RSD_r, %	5	5	17	4	8	9	3	9	12
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	0,1	0,8	0,4	0,4	0,7	1,2	0,5	2,0	0,6
Reproducibility standard deviation s_R, µg/kg	0,1	0,6	0,3	0,3	0,4	1,6	0,7	1,1	0,5
Reproducibility relative standard deviation, RSD_R, %	18	11	32	10	14	35	11	14	29
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	0,4	1,6	0,8	0,9	1,3	4,6	1,9	3,0	1,3
Recovery[a], %	65	60	70	62	65	not calculated	73	72	64
HorRat value, according to [11]	0,81	0,48	1,43	0,47	0,65	1,59	0,49	0,64	1,34

[a] Recovery values are based on the recovery of stable isotope labelled PAHs.

Annex C
(informative)

Precision data from single laboratory validation

Herbal food supplements comprise a product class that was not covered in the method validation by collaborative trial. However, it is possible to apply this analysis procedure also to this food group. Single-laboratory validation of this analysis method was performed on the determination of the four marker PAHs in a dry extract of St John's wort (*Hypericum perforatum*), containing about 6 % *Hypericum* and on a dry extract of Ginseng (*Ginseng panax*), containing about 6 % Ginseng. The latter did not contain PAHs in significant amounts, and was therefore spiked to two levels. Results of the single laboratory validation are presented in Table C.1 and Table C.2.

Table C.1 — Results from single laboratory validation for BaA and BaP

	Benz[*a*]anthracene			**Benzo[*a*]pyrene**		
	Ginseng panax	***Hypericum perforatum***	***Ginseng panax***	***Ginseng panax***	***Hypericum perforatum***	***Ginseng panax***
Mean value, $\bar{x}$, µg/kg	1,0	3,0	11,56	0,94	1,57	11,28
Repeatability SD (SD_r), µg/kg	0,05	0,29	0,28	0,06	0,11	0,39
Repeatability relative SD (RSD_r), %	4,39	8,51	2,42	6,15	5,72	3,45
Intermediate precision SD (SD_I), µg/kg	0,14	0,29	0,42	0,08	0,17	0,58
Intermediate precision relative SD (RSD_i), %	13,12	8,51	3,59	8,48	8,91	5,13
Internal reproducibility limit (2,8 x SD_i), µg/kg	0,39	0,81	1,18	0,22	0,48	1,62
Expected content, µg/kg	0,89[a]	2,98[b]	11,52[a]	0,89[a]	1,65[b]	11,52[a]

[a] Spiked.

[b] Naturally incurred (equal to assigned value from a proficiency test.

Table C.2 — Results from single laboratory validation for BbF and CHR

	Benzo[*b*]fluoranthene			Chrysene		
	Ginseng panax	***Hypericum perforatum***	***Ginseng panax***	***Ginseng panax***	***Hypericum perforatum***	***Ginseng panax***
Mean value, $\bar{x}$, µg/kg	1,16	2,92	11,92	1,09	4,24	12,11
Repeatability SD (SD_r), µg/kg	0,08	0,2	0,22	0,08	0,33	0,42
Repeatability relative SD (RSD_r), %	7,05	6,08	1,88	7,61	6,46	3,44
Intermediate precision SD (SD_i), µg/kg	0,25	0,24	0,6	0,08	0,36	0,63
Intermediate precision relative SD (RSD_i), %	21,36	7,36	5,02	7,61	7,1	5,21
Internal reproducibility limit (2,8 x SD_i), µg/kg	0,70	0,67	1,68	0,22	1,01	1,76
Expected content, µg/kg	0,89[a]	2,92[b]	11,52[a]	0,89[a]	4,07[b]	11,52[a]

[a] Spiked.

[b] Naturally incurred (equal to assigned value from a proficiency test.

Bibliography

[1] Commission 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

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