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Method of Test for Polycyclic Aromatic Hydrocarbons in Foods

1. Scope

This method is applicable for the determination of four polycyclic aromatic hydrocarbons (PAHs) including benzo[a]pyrene (BaP), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (Chr) in edible fats and oils, cacao butter, meat and meat products, aquatic animal products, infant and young child foods, dietary supplements, dried herbs, dried spices, Katsuobushi (dried bonito), cocoa shells for food ingredients, dietary supplements containing plant ingredients or plant extracts, and banana chips.

2. Method

After extraction and purification, PAHs are determined by gas chromatography/tandem mass spectrometry (GC-MS/MS).

2.1. Equipment

2.1.1. Gas chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electron ionization, EI.

2.1.1.2. Column: Select PAH capillary column, 0.15 μm, 0.25 mm × 30 m, or an equivalent product.

2.1.2. Blender.

2.1.3. Vortex mixer.

2.1.4. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder®, >1000 rpm, or an equivalent product.

2.1.5. Centrifuge: centrifugal force ≥ 5000 ×g, temperature control ≤ 15°C.

2.1.6. Ultrasonicator.

2.1.7. Solid phase extraction vacuum manifolds.

2.1.8. Nitrogen evaporator.

2.2. Chemicals

n-Hexane, residue grade;
Tetrahydrofuran, GC grade;
Methyl *tert*-butyl ether, GC grade;
Cyclohexane, GC grade;
Acetonitrile, HPLC grade;
Magnesium sulfate anhydrous, AR grade;
Sodium acetate anhydrous, AR grade;
Primary secondary amine (PSA), AR grade;
Octadecylsilane, end-capped (C18 EC), AR grade;

Deionized water, resistivity ≥ 18 MΩ•cm (at 25°C);
BaP, BaA, BbF and Chr, reference standards;
Benzo[a]pyrene-d_{12} (BaP-d_{12}), benz[a]anthracene-d_{12} (BaA-d_{12}), benzo[b]fluoranthene-d_{12} (BbF-d_{12}) and chrysene-d_{12} (Chr-d_{12}), isotope-labelled internal standards.

2.3. Apparatus

2.3.1. Centrifuge tube: 15 mL and 50 mL, PP.

2.3.2. Volumetric flask: 10 mL, 50 mL and 100 mL.

2.3.3. Solid phase extraction cartridge: HR-P, 6 mL, 500 mg, or an equivalent product; Sep-pak Silica, 6 mL, 1 g, or an equivalent product.

2.3.4. Ceramic homogenizer: Bond Elut QuEChERS P/N 5982-9313, or an equivalent product.

2.3.5. Extraction powder(Note): containing 6 g of magnesium sulfate anhydrous and 1.5 g of sodium acetate anhydrous.

2.3.6. Clean-up centrifuge tube(Note): containing 400 mg of PSA, 400 mg of C18 EC and 1200 mg of magnesium sulfate anhydrous.

2.3.7. Membrane filter: 0.22-μm, PTFE.

Note: Commercial extraction/clean-up kits can be used as needed.

2.4. Reagents

2.4.1. *n*-Hexane: methyl *tert*-butyl ether (95:5, v/v)
Mix *n*-hexane and methyl *tert*-butyl ether at the ratio of 95:5 (v/v).

2.4.2. *n*-Hexane: methyl *tert*-butyl ether (80:20, v/v)
Mix *n*-hexane and methyl *tert*-butyl ether at the ratio of 80:20 (v/v).

2.4.3. *n*-Hexane: methyl *tert*-butyl ether (50:50, v/v)
Mix n-hexane and methyl *tert*-butyl ether at the ratio of 50:50 (v/v).

2.5. Internal standard solution preparation

Transfer about 10 mg of BaP-d_{12}, BaA-d_{12}, BbF-d_{12} and Chr-d_{12} isotope-labelled internal standards accurately weighed to each 10-mL volumetric flask, dissolved and dilute to volume with *n*-hexane as the internal standard stock solutions. Store in the dark in the freezer. When to use, mix appropriate volume of each internal standard stock solution, and dilute with *n*-hexane to 5 μg/mL as the internal standard solution.

2.6. Standard solution preparation

Transfer about 10 mg of BaP, BaA, BbF and Chr reference standards

accurately weighed to each 100-mL volumetric flask, dissolve and dilute to volume with *n*-hexane as the standard stock solutions. Store in the dark in the freezer. When to use, mix appropriate volume of each standard stock solution with *n*-hexane to 0.5 μg/mL as the standard solution.

2.7. Sample solution preparation

2.7.1. Edible fats and oils

Transfer about 2 g of the liquid sample or the melted solid sample, which was melt in a water bath at 40°C, accurately weighed into a 15-mL centrifuge tube. Add 5 μL of the internal standard solution and 5 mL of *n*-hexane: methyl *tert*-butyl ether (95:5, v/v), and vortex for 15 sec. Transfer the solution into the HR-P solid phase extraction cartridge pre-rinsed with 10 mL of *n*-hexane: methyl *tert*-butyl ether (95:5, v/v). Wash the cartridge with 10 mL of *n*-hexane: methyl *tert*-butyl ether (95:5, v/v), 10 mL of *n*-hexane: methyl *tert*-butyl ether (80:20, v/v) and 10 mL of *n*-hexane: methyl *tert*-butyl ether (50:50, v/v) in order, and discard the eluents. Add 15 mL of tetrahydrofuran to the cartridge in 3 times, and collect the eluent. Evaporate the eluent to dryness by gently flushing with a stream of nitrogen at 40°C, dissolve the residue with 1 mL of *n*-hexane, and mix well. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.2. Cacao butter

Transfer about 2 g of the melted sample, which was melt in a water bath at 40°C, accurately weighed into a 15-mL centrifuge tube, and prepare the sample solution described in section 2.7.1.

2.7.3. Meat and meat products, aquatic animal products, and fruit/vegetable puree and meat puree for infant and young child

Transfer about 5 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube, add 5 mL of pre-cooled deionized water, and stand for 20 min after mixing. Add 12.5 μL of the internal standard solution, 10 mL of acetonitrile, 1 granule of a ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 4000 ×g for 5 min at 15°C, and transfer 8 mL of the supernatant to the clean-up centrifuge tube. Shake at 1000 rpm by the

high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 4000 ×g for 5 min at 15°C. Take 4 mL (a) of the supernatant, and evaporate to dryness by gently flushing with a stream of nitrogen at 40°C, dissolve the residue with 1 mL (b) of *n*-hexane, and mix well. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.4. Milk powder and cereal-based foods for infant and young child

Transfer about 5 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube, and add 5 μL of the internal standard solution and 10 mL of *n*-hexane. Vortex-mix, ultrasonicate for 10 min, centrifuge at 5000 ×g for 5 min, and collect the supernatant. Add 10 mL of *n*-hexane to the residue, and repeat the extraction procedure twice. Combine the supernatants, and evaporate to about 5 mL by gently flushing with a stream of nitrogen at 40°C. Transfer the solution into the Sep-pak Silica solid phase extraction cartridge, and collect the eluent. Add 10 mL of *n*-hexane to the cartridge, and combine the eluents. Evaporate to dryness by gently flushing with a stream of nitrogen at 40°C, dissolve the residue with 1 mL of *n*-hexane, and mix well. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.5. Royal jelly, dietary supplements containing spirulina, dried herbs, dried spices, Katsuobushi (dried bonito), and dietary supplements containing plant ingredients or plant extracts

Transfer about 0.5 g of the homogenized sample accurately weighed into a 15-mL centrifuge tube, and add 5 μL of the internal standard solution and 4 mL of cyclohexane. Vortex-mix, ultrasonicate for 10 min, centrifuge at 5000 ×g for 5 min, and collect the supernatant. Add 4 mL of cyclohexane to the residue, and repeat the extraction procedure twice. Combine the supernatants, and evaporate to about 5 mL by gently flushing with a stream of nitrogen at 40°C. Transfer the solution into the Sep-pak Silica solid phase extraction cartridge, and collect the eluent. Add 10 mL of cyclohexane to the cartridge, and combine the eluents. Evaporate to dryness by gently flushing with a stream of nitrogen at 40°C, dissolve the residue with 1 mL of *n*-hexane, and mix well. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.6. Banana chips, and cocoa shells for food ingredients

Transfer about 1 g of the homogenized sample accurately weighed into a 15-mL centrifuge tube, and prepare the sample solution described in section 2.7.5.

2.8. Matrix-matched calibration curve

2.8.1. Edible fats and oils

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.7.1 to obtain the eluent from the solid phase extraction cartridge. Evaporate to dryness by gently flushing with a stream of nitrogen, separately add 2 - 100 μL of the standard solution and 5 μL of the internal standard solution, and dilute with appropriate amount of *n*-hexane to 1 mL as the matrix-matched standard solutions. Operate GC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of each PAH by the ratios of the peak area of each PAH to that of the internal standard vs. the added concentrations in the range of 1 - 50 ng/mL.

GC-MS/MS operating conditions(Note):

Column: Select PAH capillary column, 0.15 μm, 0.25 mm × 30 m.

Oven temperature:

Initial temperature: 50°C;

Temperature rising rate: 10°C/min;

Middle temperature 1: 200°C, 2 min;

Temperature rising rate: 10°C/min;

Middle temperature 2: 250°C, 10 min;

Temperature rising rate: 10°C/min;

Middle temperature 3: 270°C, 9 min;

Temperature rising rate: 30°C/min;

Final temperature: 320°C, 15 min.

Carrier gas and flow rate: helium, 1 mL/min.

Injector temperature: 320°C.

Injection mode: splitless.

Injection volume: 2 μL.

Interface temperature: 280°C.

Ion source temperature: 340°C.

Ion source: EI, 70 eV.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair and collision energy are shown as follows.

Analyte	Ion pair Precursor ion (*m*/*z*) > product ion (*m*/*z*)	Collision energy (eV)
Benz[a]anthracene	228 > 226*	35
	113 > 112	15
	228 > 227	20
Benzo[b]fluoranthene	252 > 250*	40
	125 > 124	15
	126 > 113	15
Benzo[a]pyrene	252 > 250*	40
	125 > 124	15
	126 > 113	15
Chrysene	228 > 226*	35
	228 > 227	20
	113 > 112	15
Benz[a]anthracene-d_{12} (I.S.)	240 > 236	35
Benzo[b]fluoranthene-d_{12} (I.S.)	264 > 260	35
Benzo[a]pyrene-d_{12} (I.S.)	264 > 260	40
Chrysene-d_{12} (I.S.)	240 > 236	35

*The quantitative ion, and a qualitative ion pair can be selected based on the matrix condition.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.8.2. Cacao butter

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.7.2 to obtain the eluent from the solid phase extraction cartridge. Evaporate to dryness by gently flushing with a stream of nitrogen, separately add 10 - 100 μL of the standard solution and 5 μL of the internal standard solution, and dilute with appropriate amount of *n*-hexane to 1 mL as the matrix-matched standard solutions. Operate GC-MS/MS according to the conditions described in section 2.8.1. Establish the matrix-matched calibration curve of each PAH by the ratios of the peak area of each PAH to that of the internal standard vs. the added

concentrations in the range of 5 - 50 ng/mL.

2.8.3. Meat and meat products, aquatic animal products, and fruit/vegetable puree and meat puree for infant and young child

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.7.3 to obtain the supernatant after the clean-up procedure. Take 4 mL of the supernatant, and evaporate to dryness by gently flushing with a stream of nitrogen. Separately add 2 - 100 μL of the standard solution and 5 μL of the internal standard solution, and dilute with appropriate amount of *n*-hexane to 1 mL as the matrix-matched standard solutions. Operate GC-MS/MS according to the conditions described in section 2.8.1. Establish the matrix-matched calibration curve of each PAH by the ratios of the peak area of each PAH to that of the internal standard vs. the added concentrations in the range of 1 - 50 ng/mL.

2.8.4. Milk powder and cereal-based foods for infant and young child

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.7.4 to obtain the eluent from the solid phase extraction cartridge. Evaporate to dryness by gently flushing with a stream of nitrogen, separately add 2 - 100 μL of the standard solution and 5 μL of the internal standard solution, and dilute with appropriate amount of *n*-hexane to 1 mL as the matrix-matched standard solutions. Operate GC-MS/MS according to the conditions described in section 2.8.1. Establish the matrix-matched calibration curve of each PAH by the ratios of the peak area of each PAH to that of the internal standard vs. the added concentrations in the range of 1 - 50 ng/mL.

2.8.5. Royal jelly, dietary supplements containing spirulina, dried herbs, dried spices, Katsuobushi (dried bonito), and dietary supplements containing plant ingredients or plant extracts

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.7.5 to obtain the eluent from the solid phase extraction cartridge. Evaporate to dryness by gently flushing with a stream of nitrogen, separately add 5 - 100 μL of the standard solution and 5 μL of the internal standard solution, and dilute with appropriate amount of *n*-hexane to 1 mL as the matrix-matched standard solutions. Operate

GC-MS/MS according to the conditions described in section 2.8.1. Establish the matrix-matched calibration curve of each PAH by the ratios of the peak area of each PAH to that of the internal standard vs. the added concentrations in the range of 2.5 - 50 ng/mL.

2.8.6. Banana chips, and cocoa shells for food ingredients

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.7.6 to obtain the eluent from the solid phase extraction cartridge eluent. Evaporate to dryness by gently flushing with a stream of nitrogen, separately add 2 - 100 μL of the standard solution and 5 μL of the internal standard solution, and dilute with appropriate amount of *n*-hexane to 1 mL as the matrix-matched standard solutions. Operate GC-MS/MS according to the conditions described in section 2.8.1. Establish the matrix-matched calibration curve of each PAH by the ratios of the peak area of each PAH to that of the internal standard vs. the added concentrations in the range of 1 - 50 ng/mL.

2.9. Identification and quantitation

Accurately inject 2 μL of the sample solution and the matrix-matched standard solutions into GC-MS/MS separately, and operate according to the conditions described in section 2.8. Identify each PAH based on the retention time and the relative ion intensities(Note). Calculated the amount (μg/kg) of each PAH in the sample by the following formula:

2.9.1. Edible fats and oils, cacao butter, milk powder and cereal-based foods for infant and young child, royal jelly, dietary supplements containing spirulina, dried herbs, dried spices, Katsuobushi (dried bonito), dietary supplements containing plant ingredients or plant extracts, banana chips, and cocoa shells for food ingredients

$$\text{The amount of each PAH in the sample (μg/kg)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each PAH in the sample solution calculated by matrix-matched calibration curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

2.9.2. Meat and meat products, aquatic animal products, and fruit/vegetable

puree and meat puree for infant and young child

$$\text{The amount of each PAH in the sample (μg/kg)} = \frac{C \times V}{M} \times F$$

Where,

C: the concentration of each PAH in the sample solution calculated by matrix-matched calibration curve (ng/mL)

V: the volume of extraction solution (10 mL)

M: the weight of the sample (g)

F: the concentration factor, b/a (1/4)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ionic intensity (%)	Tolerance (%)
> 50	± 20
> 20~50	± 25
> 10~20	± 30
≤ 10	± 50

Remark

1. The limit of quantification (LOQ) for each PAH in the sample is as follows:

Sample	LOQ for each PAH (μg/kg)
Edible fats and oils, meat and meat products, aquatic animal products, and fruit/vegetable puree and meat puree for infant and young child	0.5
Cacao butter	2.5
Milk powder and cereal-based foods for infant and young child	0.2
Royal jelly, dietary supplements containing spirulina, dried herbs, dried spices, Katsuobushi (dried bonito), and dietary supplements containing plant ingredients or plant extracts	5

Banana chips, and cocoa shells for as food ingredients	1

2. The Select PAH column used in this method cannot separate chrysene and its isomer, triphenylene, completely. If the sample contains the above two compounds at the same time and the content of four PAHs exceeds the indicative value, the ZB-PAH-SeleCT column (0.14 μm, 0.18 mm × 40 m) or an equivalent product is recommended to use to separate the two compounds for further quantitation.
3. Further validation should be performed when interfering compounds are found in the samples.

Reference

1. Forsberg, N. D., Wilson, G. R. and Anderson, K. A. 2011. Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive SPE and GC-MS. J. Agric. Food Chem. 59: 8108-8116.
2. Rey-Salgueiro, L., Martínez-Carballo, E., García-Falcón, M. S., González-Barreiro, C. and Simal-Gándara, J. 2009. Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods. Food Chem. 115: 814-819.
3. Albero, B., Sánchez-Brunete, C. and Tadeo, J. L. 2003. Determination of polycyclic aromatic hydrocarbons in honey by matrix solid-phase dispersion and gas chromatography/mass spectrometry. J. AOAC Int. 86: 576-582.

Reference chromatogram

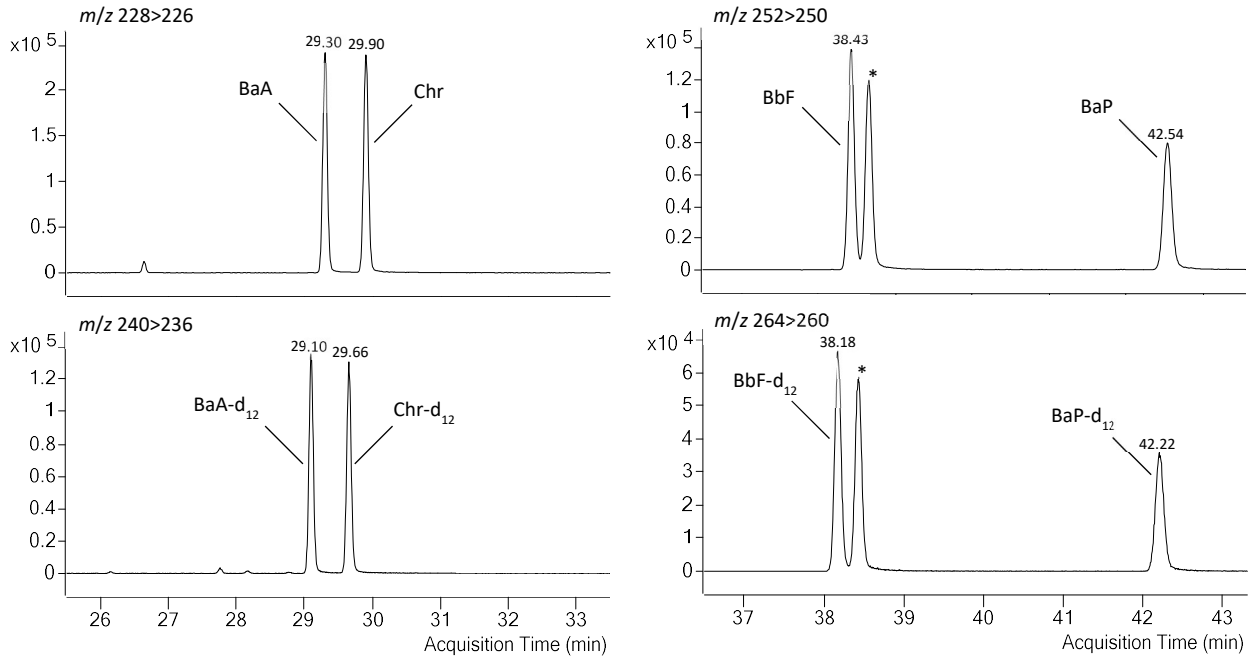

Figure. MRM chromatograms of benz[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, chrysene and their isotope-labelled internal standards analyzed by GC-MS/MS.

*The chromatographic peaks of another PAH and its isotope-labelled internal standard.