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Method of Test for Polycyclic Aromatic Hydrocarbons in Cosmetics

1. Scope

This method is applicable to the determination of anthracene (Ant), benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), chrysene (Chr), dibenzo[a,h]anthracene (DBA) and naphthalene (Nap) in cosmetics.

2. Method

After extraction, analytes 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：DB-EUPAH, 0.14 μm, 0.18 mm i.d. × 20 m, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Acetone, HPLC grade;

Hexane, HPLC grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);

Ant, BaA, BaP, BbF, BjF, BkF, Chr, DBA, and Nap, reference standards;

anthracene-d_{10} (Ant-d_{10}), benzo[a]anthracene-d_{12} (BaA-d_{12}), benzo[a]pyrene-d_{12} (BaP-d_{12}), benzo[b]fluoranthene-d_{12} (BbF-d_{12}), benzo[k]fluoranthene-d_{12} (BkF-d_{12}), chrysene-d_{12} (Chr-d_{12}), dibenzo[a,h]anthracene-d_{14} (DBA-d_{14}), and naphthalene-d_8 (Nap-d_8), isotope labeled internal standards.

2.3. Apparatus：

2.3.1. Volumetric flask：10 mL and 20 mL.

2.3.2. Membrane filter：0.22 μm, Nylon.

2.4. Acetone：hexane (50:50, v/v) solution

Mix acetone and hexane at the ratio of 50：50 (v/v).

2.5. Internal standard solution preparation

Transfer about 10 mg of Ant-d_{10}, BaA-d_{12}, BaP-d_{12}, BbF-d_{12}, BkF-d_{12} Chr-d_{12}, DBA-d_{14}, and Nap-d_8 internal standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with acetone: hexane (50: 50, v/v) solution to volume as the internal standard stock solutions. Store in the refrigerator and protect from light. When to use, mix appropriate amount of

each internal standard stock solutions, and dilute with acetone: hexane (50:50, v/v) solution to 200 ng/mL as the internal standard solution.

2.6. Standard solution preparation

Transfer about 20 mg of Ant, BaA, BaP, BbF, BjF, BkF, Chr, DBA, and Nap reference standards accurately weighed to each 20-mL volumetric flask, dissolve and dilute with acetone: hexane (50:50, v/v) solution to volume as the standard stock solutions. Store in the refrigerator and protect from light. When to use, mix appropriate amount of each standard stock solution, and dilute with acetone: hexane (50:50, v/v) solution to 0.25-10 ng/mL for Ant, BaA, BaP and Chr,to 0.5-10 ng/mL for BbF, BjF, BkF, DBA and Nap (Containing 1 ng/mL isotope labeled internal standards), as the standard solutions.

2.7. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20-mL volumetric flask, add10 mL of acetone: hexane (50:50, v/v) solution, and ultrasonicate for 30 mins. Dilute to volume with acetone: hexane (50:50, v/v) solution as the sample stock solution. Dilute sample stock solution with acetone: hexane (50:50, v/v) solution 10 times, andfilter with a membrane filter.Take the filtrate as the sample solution.

2.8. Standard curve preparation：

Accurately inject 2 μL of the standard solutions into GC-MS/MS separately. Operate GC-MS/MS according to the following conditions. Establish the standard curve of each polycyclic aromatic hydrocarbon by the ratios of the peak area of each polycyclic aromatic hydrocarbon to that of the isotope labeled internal standard vs. the concentrations of each polycyclic aromatic hydrocarbon.

GC-MS/MS operating conditions(Note):

Column: DB-EUPAH capillary column, 0.14 μm, 0.18 mm i.d. × 20 m.

Column temperature: initial temperature: 50℃, 2 min;
temperature gradientrate: 25℃/min;
middle temperature 1: 150℃, hold for 3 min;
temperature gradient rate: 5℃/min;
middle temperature 2: 165℃, hold for 3 min;
temperature gradient rate: 10℃/min;
middle temperature 3: 175℃, hold for 5 min;

temperature gradient rate: 25℃/min;
middle temperature 4: 225℃, hold for 5 min;
temperature gradient rate: 20℃/min;
middle temperature 5: 265℃, hold for 10 min;
temperature gradient rate: 5℃/min;
middle temperature 6: 300℃, hold for 6 min;
temperature gradient rate: 10℃/min;
final temperature: 320℃, hold for 5 min.

Carrier gas and flow rate: helium, 2 mL/min.

Injector temperature: 340℃.

Inlet mode: splitless.

Injection volume: 2 μL.

Interface temperature: 340℃.

Ion source temperature: 340℃.

Ionization mode: 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)	Internal standard
Ant	178 > 176*	35	Ant-d_{10}
	178 > 152	25	
BaA	228 > 226*	35	BaA-d_{12}
	113 > 112	15	
BaP	252 > 250*	40	BaP-d_{12}
	126 > 113	15	
BbF	252 > 250*	40	BbF-d_{12}
	126 > 113	15	
BjF	252 > 250*	40	BbF-d_{12}
	125> 124	15	
BkF	252 > 250*	40	BkF-d_{12}
	126 > 113	15	
Chr	228 > 226*	20	Chr-d_{12}
	228 > 227	20	

DBA	278 > 276*	45	DBA-d_{14}
	139 > 138	15	
Nap	128 > 102*	20	Nap-d_8
	128 > 78	25	
Ant-d_{10} (I.S.)	188 > 160	25	-
BaA-d_{12} (I.S.)	240 > 236	35	-
BaP-d_{12} (I.S.)	264 > 260	40	-
BbF-d_{12} (I.S.)	264 > 260	35	-
BkF-d_{12} (I.S.)	264 > 260	35	-
Chr-d_{12} (I.S.)	240 > 236	20	-
DBA-d_{14} (I.S.)	292 > 288	30	-
Nap-d_8 (I.S.)	136 > 108	15	-

*The quantitative ion pair

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

2.9. Identification and quantification

Accurately inject 2 μL of the sample solution and the standard solutions into GC-MS/MS separately, and operate according to the conditions described in section 2.8. Identify each polycyclic aromatic hydrocarbon based on the retention time and the relative ion intensities(note). Calculate the amount of each polycyclic aromatic hydrocarbon in the sample by the following formula:

The amount of each polycyclic aromatic hydrocarbon in the sample (ppm) =

$$\frac{C \times V \times F}{M} \times 10^{-3}$$

Where,

C: the concentration of each polycyclic aromatic hydrocarbon in the sample solution calculated by the calibration curve (ng/mL).

V: the final make-up volume of samples (20 mL)

M: the weight of the sample (g)

F: Dilution factor (10)

Note: 1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤100%). Maximum permitted tolerances for relative ion intensities by GC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

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

Remark

1. The limits of quantification (LOQs) are 0.05 ppm for Ant, BaA, BaP and Chr, and 0.1 ppm for BbF, BjF, BkF, DBA and Nap.
2. Further validation should be performed when interference appearin samples.

References

1. Taiwan Food and Drug Administration, Ministry of Health and Welfare. Method of test for polycyclic aromatic hydrocarbons in Foods. (TFDAO0030.01). Published on Mar 2, 2020.
2. Wang, S. W., Hsu, K. H., Huang, S. C., Tseng, S. H., Wang, D. Y. and Cheng, H. F. 2019. Determination of polycyclic aromatic hydrocarbons (PAHs) in cosmetic products by gas chromatography-tandem mass spectrometry. J. Food Drug Anal. 27: 815-824.

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Reference chromatogram

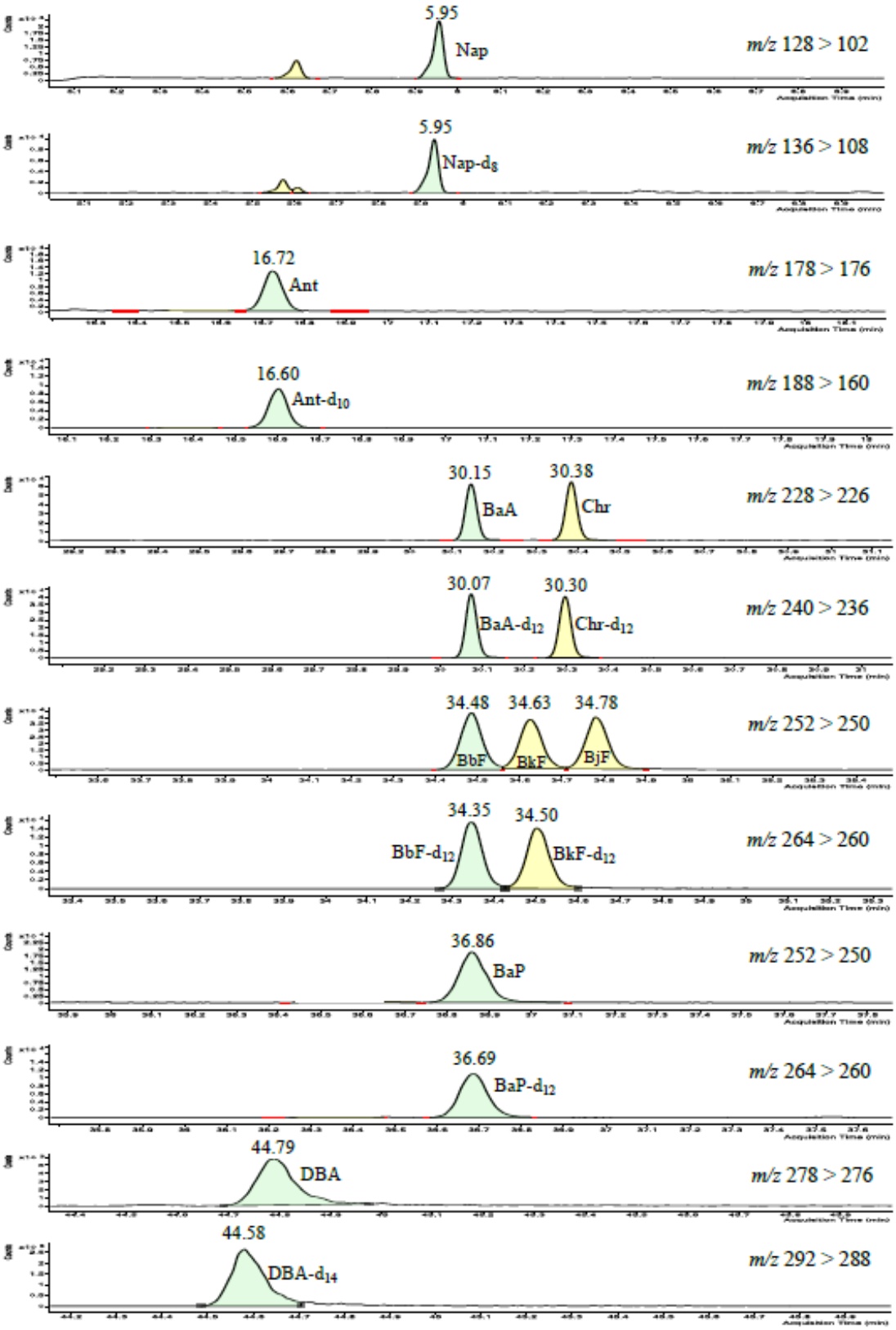

Figure. MRM chromatograms of polycyclic aromatic hydrocarbon standards analyzed by GC-MS/MS.