GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Determination of polycyclic aromatic hydrocarbons in foods

食品安全国家标准

食品中多环芳烃的测定

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China Food and Drug Administration.

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National food safety standard - Determination of polycyclic aromatic hydrocarbons in foods

1 Scope

This standard specifies the liquid chromatography for determining polycyclic aromatic hydrocarbons in food (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[α]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[α]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[α,h]anthracene and benzo[g,h,i]perylene), as well as the gas chromatography-mass spectrometry for determining the polycyclic aromatic hydrocarbons in foods (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[α]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[α]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[α,h]anthracene and benzo[g,h,i]perylene).

This standard applies to the determination of polycyclic aromatic hydrocarbons in foods.

Method I - High-performance liquid chromatography

2 Principle

The polycyclic aromatic hydrocarbons in the specimen are extracted by the use of an organic solvent. The extract is concentrated to near dryness, dissolved in a solvent, purified by the use of PSA (N-propylethylenediamine) and C_{18} solid-phase extraction filler or by the use of Florisil solid-phase extraction column. After concentration and making it reach to a certain volume, it is separated by the high-performance liquid chromatography. The fluorescence intensity of various polycyclic aromatic hydrocarbons at different excitation wavelengths and emission wavelengths is determined. It is quantified by external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade, the water is the grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN): Chromatographically pure.

3.1.2 n-Hexane (C_6H_{14}): Chromatographically pure.

3.1.3 Dichloromethane (CH_2Cl_2): Chromatographically pure.

3.1.4 Diatomaceous earth: Chromatographically pure.

3.1.5 Magnesium sulfate ($MgSO_4$): Excellent grade.

3.1.6 N-propylethylenediamine (PSA): Particle size 40 μm.

3.1.7 Tai-sealed C_{18} solid-phase extraction packing: Particle size 40 μm ~ 63 μm.

3.1.8 Florisil solid-phase extraction column: 500 mg, 3 mL.

3.1.9 Organic phase microporous membrane: 0.22 μm.

3.2 Preparation of reagent

3.2.1 n-hexane-dichloromethane mixed solution (1 + 1): TAKE 500 mL of n-hexane; ADD 500 mL of dichloromethane; MIX it uniformly.

3.2.2 Acetonitrile-saturated n-hexane: TAKE 800 mL of n-hexane; ADD 200 mL of acetonitrile; SHAKE to mix it uniformly; LET it be standing for layering. The upper n-hexane layer is acetonitrile-saturated n-hexane.

3.3 Standard substance

Polycyclic aromatic hydrocarbons (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[α]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[α]pyrene, indeno [1,2,3-c,d]pyrene, dibenzo[α,h]anthracene and benzo[g,h,i]perylene) certified standard solution (200 μg/mL), which is preserved at -18 °C.

Warning - Polycyclic aromatic hydrocarbons are known to be carcinogenic, teratogenic, mutagenic. The carcinogenicity increases with increasing number of benzene rings. Special attention shall be paid to safety protection during the determination. The determination shall be carried out in a fume hood, it shall wear gloves, to minimize exposure.

3.4 Preparation of standard solution

3.4.1 Polycyclic aromatic hydrocarbon standard intermediate solution (1000 ng/mL): PIPETTE 0.5 mL of polycyclic aromatic hydrocarbon standard solution; USE acetonitrile to make its volume reach to 100 mL. PRESERVE it at -18 °C.

a) ADD 10 mL of n-hexane; VORTEX it for 30 s; then PLACE it in a 40 °C water bath for ultrasonic for 30 min; CENTRIFUGE it at 4500 r/min for 5 min; TAKE the supernatant in a glass centrifuge tube B; USE 10 mL of n-hexane to repeat extraction one time for the lower part of the centrifuge tube A; COMBINE the extract into the centrifuge tube B; PERFORM nitrogen blowing (temperature control below 35 °C) to remove the reagent; BLOW it almost dry.

b) In the centrifuge tube B, ADD 4 mL of acetonitrile; VORTEX it for 30 s; then ADD 900 mg of magnesium sulfate, 100 mg of PSA and 100 mg of C18 packing; VORTEX it mix it for 30 s; CENTRIFUGE at 4500 r/min for 3 min; TAKE the supernatant into a 10 mL glass graduated centrifuge tube C; USE 2 mL of acetonitrile to repeat extraction once for the lower part of the centrifuge tube B; COMBINE the extract into the centrifuge tube C; PERFORM nitrogen blowing to the reagent to make it reach to about 1 mL; USE acetonitrile to make its volume reach to about 1 mL; MIX it uniformly; MAKE it pass the 0.22 μm organic-phase microporous membrane, to obtain the sample solution to be determined.

5.2.2 Foods such as aquatic products, meat and vegetables

WEIGH 2 g ~ 5 g (accurate to 0.01 g) of specimen in a 50 mL stoppered glass centrifuge tube A; ADD 1 g ~ 5 g of diatomaceous earth; USE a glass rod to stir it uniformly; MAKE treatment according to steps a) and b) in 5.2.1 subsequently, to obtain the sample solution to be determined.

5.2.3 High-fat foods or animal & vegetable oils

WEIGH 1 g ~ 4 g (accurate to 0.01 g) of specimen in a 50 mL stoppered glass centrifuge tube A; USE the procedures below to make treatment:

a) ADD 20 mL of acetonitrile and 10 mL of acetonitrile-saturated n-hexane; VORTEX it for 30 s; PLACE it in a 40 °C water bath for ultrasonic for 30 min; after shaking it uniformly, FREEZE and CENTRIFUGE it at 4500 r/min (-4 °C) for 5 min; PIPETTE the lower layer of acetonitrile in a 100 mL heart-shaped bottle; USE 20 mL of acetonitrile to make repeated extraction once for the solution in the centrifuge tube A; COMBINE the extract in the heart-shaped bottle; MAKE it subject to 35 °C rotary evaporation under reduced pressure to near dryness. ADD 5 mL of n-hexane; VORTEX it for 30 s to dissolve it.

b) Sequentially USE 5 mL of dichloromethane and 10 mL of n-hexane to activate the Florisil solid-phase extraction column; TRANSFER all the 5 mL extracted sample which is obtained from a) into the Florisil solid-phase extraction column; then USE 5 mL of n-hexane to wash the heart-shaped bottle; COMBINE the washing solution into the column. USE 8 mL of n-

12.2 Extraction of specimen

Same as 5.2.

12.3 Blank test

The blank test uses the same analytical procedure as the test except that no specimen is added.

12.4 Reference conditions for gas chromatography-mass spectrometry

a) Column: DB-5 MS, the column length is 30 m, the inner diameter is 0.25 mm, the film thickness is 0.25 μm, or column of equivalent performance.

b) Column's temperature program: The initial temperature is 90 °C, rise the temperature at 20 °C/min to 320 °C, maintain this temperature for 2 min;

c) Sample inlet's temperature: 250 °C;

d) Chromatography-mass interface's temperature: 280 °C;

e) Ion source temperature: 230 °C;

f) Carrier gas: helium, purity ≥ 99.999%, 1.0 mL/min;

g) Ionization mode: EI;

h) Ionization energy: 70 eV;

i) Mass scanning range: 50 amu ~ 450 amu;

j) Method of determination: Selective ion monitoring mode;

k) Injection method: non-split injection; open the valve after 2.0 min;

l) Sample injection volume: 1.0 μL;

m) Solvent delay: 3 min.

12.5 Production of standard curves

Respectively INJECT the standard series working solution into the gas chromatography-mass spectrometer, to determine the corresponding peak area. USE the mass concentration of the standard working solution as the abscissa and the peak area as the ordinate, to draw the standard curve.

12.6 Determination of sample solution

INJECT the sample solution to be determined into the gas chromatograph-mass spectrometer, to determine the corresponding peak area. According to

13 Expression of analytical results

The content of polycyclic aromatic hydrocarbons in the sample, X_i, is calculated according to formula (2):

$$X_i = \frac{\rho_i \times V \times 1\,000}{m \times 1\,000} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (2)$$

Where:

X_i - The content of polycyclic aromatic hydrocarbons in the specimen, in micrograms per kilogram (μg/kg);

ρ_i - The concentration of polycyclic aromatic hydrocarbons i in the sample solution to be determined which is calculated according to the standard curve, in nanograms per milliliter (ng/mL);

V - The final constant volume of the sample solution to be determined, in milliliters (mL);

1000 - Unit conversion;

m - The mass of the specimen, in grams (g).

When the content is greater than or equal to 10 μg/kg, it retains three significant figures; when the content is less than 10 μg/kg, it retains two significant figures.

Note: The calculation result shall be deducted from the blank value.

14 Precision

The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 20% of the arithmetic mean.

15 Others

When the sample amount is 4 k and the constant volume is 1 mL, the detection limit and limit of quantification of the method are as shown in Table 5.

------ The End ------