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Animal and vegetable fats and oils – Determination of benzo (α) pyrene – Reverse-phase high performance liquid chromatography method

动植物油脂 苯并（α）芘的测定

反相高效液相色谱法

(ISO 15302:2007, MOD)

Issued on: November 04, 2008 **Implemented on: January 20, 2009**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine;

Standardization Administration of PRC.

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Foreword

This standard, through modification, adopts the ISO 15302:2007 Animal and vegetable fats and oils – Determination of benzo (α) pyrene – Reverse-phase high performance liquid chromatography method (English version).

As compared with ISO 15302:2007, the main technical differences are as follows:

- DELETE the noun terminology;
- Appropriately MODIFY the instrument and equipment;
- ADD the measurement methods for the alumina activity;
- CHANGE the elution reagent petroleum ether (40 °C ~ 60 °C) or n-hexane into petroleum ether (30 °C ~ 60 °C) or n-hexane;
- CHANGE the constant volume of the tested solution from 250 μL to 100 μL; CHANGE the sample injection volume from 50 μL to 10 μL;
- CHANGE the internal standard method quantitative calculation into the external standard quantitative calculation.

Annexes A, B, C and D of this standard are informative.

This standard was proposed by the State Grain Administration.

This standard shall be under the jurisdiction of the National Grain and Oil Standardization Technical Committee.

The drafting organizations of this standard: Dalian City Product Quality Supervision and Inspection Institute, the State Food Quality Supervision and Inspection Center, Dalian Standard Detection Technology Research Center.

The main drafters of this standard: Dong Guangbin, Zheng Shunli, Wang Chunyan, Li Peng, Pan Wei, Jiang Jun, Li Haiyan, Gu Xinrong, Guan Cheng, Mao Xiqin, Yong Yanhua, Liu Zhizhang, Zhang Xiaofan.

Animal and vegetable fats and oils – Determination of benzo(α)pyrene – Reverse-phase high performance liquid chromatography method

1 Scope

This standard specifies the principles, reagents and materials, apparatus and equipment, sampling method, sample preparation, operation procedures, test results presentation, precision and so on for the determination of benzo (a) pyrene in animal and vegetable fats and oils through the reverse-phase high performance liquid chromatography method.

This standard applies to animal and vegetable fats and oils.

The minimum detection limit of this standard method is 0.1 μg/kg.

The measurement range of this standard is 0.1 μg/kg ~ 50 μg/kg.

2 Normative references

The provisions in following documents become the provisions of this standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this standard; however, parties who reach an agreement based on this standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6379.2 Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic methods for the determination of repeatability and reproducibility of a standard measurement method (GB/T 6379.2-2004, ISO 5725-2:1994, IDT);

GB/T 6682 Water for analytical laboratory use – Specification and test methods (GB/T 6682-2008, ISO 3696:1987, MOD);

GB/T 15687 Oils and fats – Preparation of test sample (GB/T 15687-1995, eqv ISO 661:1989)

4.9 benzo (a) pyrene standard sample: CAS No.: 50-32-8, AND its purity is not less than 99.0%.

Warning - benzo (a) pyrene is a known carcinogen, so during measurement it shall pay special attention to safety protection. The measurement shall be conducted in a fume hood, AND the operator shall wear gloves and minimize exposure.

4.10 Benzo (a) pyrene standard stock solution: accurately WEIGH 12.5 mg of benzo (a) pyrene (4.9); PLACE it into a 25 mL volumetric flask; USE toluene (4.6) to dissolve AND make it reach to volume. The content of benzo (a) pyrene in this solution is about 0.5 mg/mL. PRESERVE it at 4 °C in dark, which can maintain stable for at least 6 months.

4.11 Standard working solution: USE the benzo (a) pyrene standard stock solution (4.10) to respectively prepare two kinds of standard solutions, the benzo (a) pyrene content of which is about 0.2 μg/mL and 0.01 μg/mL respectively .

5 Instruments and equipment

5.1 Glass column (SEE Appendix B): equipped with sintered glass mats and Teflon taps.

5.2 Constant temperature water bath.

5.3 Rotary evaporator.

5.4 HPLC: If auto-samplers are used, the sample loop should be rinsed with acetonitrile in the sequence.

5.5 Glass vial: Approximately 1 mL with a sealable lid.

6 Sampling method

The samples received by the laboratory shall be representative, AND the samples shall not be damaged or altered during transport and storage.

This standard does not specify the sampling method, AND it is recommended to use the sampling method as specified in GB/T 5524.

b) Column: Polycyclic aromatic hydrocarbon analysis column, 4.6 mm × 250 mm;

c) Sample injection volume: 10 μL;

d) Mobile phase: acetonitrile : water = 880 :120 (volume ratio);

e) Flow rate: 1.0 mL/min;

f) Fluorescence detector: emission wavelength: 406 nm (slit: 10 nm), excitation wavelength: 384 nm (slit: 10 nm).

8.2.2 Drawing of standard curve

DILUTE the standard working solution (4.11) to five solutions of different concentrations; when the sample injection volume of each solution is 10μL, the mass of benzo (a) pyrene is 0.004 ng, 0.008 ng, 0.04 ng, 0.2 ng, 0.4 ng. Based on the integrated area of the peak, DRAW the 5-point calibration curve. As for the chromatogram, SEE Appendix C.

8.3 Sample analysis

8.3.1 In the glass vial containing the tested sample (8.1), INJECT 100 μL of the acetonitrile and tetrahydrofuran mixed solution (4.5). Carefully SWIRL to dissolve the residues, and AVOID the vial tap from contacting with the solvent. USE the standard curve (8.2.2) to quantify the benzo (a) pyrene in the range of 0.1 μg/kg ~ 50 μg/kg. With respect to the sample for which the benzo (a) pyrene content is more than 10 μg/kg, it is allowed to use the acetonitrile and tetrahydrofuran mixed solution (4.5) to dilute it OR otherwise reduce the sample injection volume.

8.3.2 INJECT 10 μL of the sample solution into a liquid chromatograph for measurement. ENSURE that no more than 1.5 mg of dissolved residue is injected into the column. If it exceeds 1.5 mg, it shall use the tetrahydrofuran (4.4) to dilute OR otherwise purify it again.

9 Presentation of test results

The benzo (a) pyrene content is calculated using the equation (1):

$$w = \frac{c \times V}{m} \qquad \cdots\cdots(1)$$

Where:

Appendix A

(Informative)

Brockmann alumina activity measurement methods

A.1 Definition of activity

Depending on the water content, the neutral alumina can be divided into five activity levels. Based on Brockmann's definitions, the level I alumina is the alumina which is fired at 450 °C for 12 h; whilst the level II, III, IV and V alumina is produced by respectively adding 3%, 6%, 10% and 15% of water into the level I alumina.

A.2 Measurement

A.2.1 Chromatographic column: glass column, Φ 15 mm × H 150 mm, the lower part is cushioned with a little absorbent cotton, FILL 50 mm height alumina; MAKE it tight.

A.2.2 Reagents: benzene + petroleum ether (1 +4).

A.2.3 Activity test solution preparation: based on the following combinations, respectively TAKE 20 mg of each kind of azo dye; USE the reagent (A.2.2) to set the volume in a 50mL volumetric flask, to prepare the corresponding dye mixed solution.

The first pair (I): azobenzene (AB) and p-methoxazobenzene (MAB);

The second pair (II): MAB and Sudan I (Sudan I, S I);

The third pair (III): Sudan I and Sudan III (Sudan III, S III);

The fourth pair (IV): Sudan III and p-aminoazobenzene (AAB);

The fifth pair (V): AAB and p-hydroxyazobenzene (HAB).

A.2.4 Respectively TAKE 10 mL of the dye mixture solution I, II, III, IV, and V; slowly ADD them into five alumina chromatography columns; after they are empty, ADD another 20 mL of reagent (A.2.2); after it is emptied, OBSERVE the movement of the colored dye along the column, in order to determine the alumina activity, as shown in Figure A.1. If the first pair of dye solution is completely adsorbed on the column, the alumina activity is level I; if the MAB

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