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National food safety standard - Food contact materials and products - Determination of migration and release of N-nitrosamines

食品安全国家标准 食品接触材料及制品 N-亚硝胺类化合物迁移量和释放量的测定

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National food safety standard - Food contact materials and products - Determination of migration and release of N-nitrosamines

1 Scope

This Standard specifies the method for determining the migration and release of N-nitrosamine compounds and N-nitrosamine derivatives in food contact materials and articles.

The first part of this Standard is applicable to the determination of the migration of 15 N-nitrosamine compounds, including N-nitroso-dimethylamine, N-nitroso-N-methylethylamine, N-nitrosodiethylamine, N-nitrosodiisopropylamine, N-nitrosodi-n-propylamine, N-nitrosodiisobutylamine, N-nitrosodi-n-butylamine, N-nitrosopiperidine, N-nitrosopyrrolidine, N-nitrosomorpholine, N-nitroso-N-ethylaniline, N-nitroso-N-methylaniline, N-nitrosodiisononylamine, N-nitrosodicyclohexylamine, and N-nitrosodibenzylamine, in rubber materials and products for food contact. It is applicable to the determination of the migration of the above 15 N-nitrosamine products in rubber materials and products for food contact. The second part of this Standard is applicable to the determination of the release of the above 15 N-nitrosamine compounds in pacifiers. It is also applicable to the determination of the release of the above 15 N-nitrosamine products in pacifiers.

Part 1 -- Determination of migration of N-nitrosamine compounds and N-nitrosamine products

Method One -- Gas chromatography-mass spectrometry

2 Principle

The N-nitrosamine compounds in the soaking solution obtained from the migration test are extracted with dichloromethane under alkaline conditions, concentrated, and determined by gas chromatography-mass spectrometry (GC-MS). The external standard method is used for quantification to obtain the migration amount of N-nitrosamine compounds directly migrated.

The N-nitrosamine-derived products in the immersion solution obtained in the

migration test react with nitrite in artificial saliva under acidic conditions to generate N-nitrosamine compounds. The N-nitrosamine compounds generated by the reaction and the N-nitrosamine compounds directly migrated are simultaneously determined according to the above steps to obtain the sum of the migration amounts of the N-nitrosamine-derived products and the N-nitrosamine compounds directly migrated. The migration amount of a single N-nitrosamine-derived product is calculated by subtracting the migration amount of the corresponding N-nitrosamine directly migrated from the sum of the measured migration amounts of the two.

3 Reagents and materials

Unless otherwise specified, all reagents used in this method are analytically pure and water is Grade one water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Dichloromethane (CH_2Cl_2): chromatographically pure.

3.1.2 Concentrated hydrochloric acid (HCl).

3.1.3 Sodium hydroxide (NaOH).

3.1.4 Anhydrous ethanol (C_2H_6O): chromatographically pure.

3.1.5 Glacial acetic acid ($C_2H_4O_2$): guaranteed reagent.

3.1.6 Sodium bicarbonate ($NaHCO_3$).

3.1.7 Sodium chloride (NaCl).

3.1.8 Potassium carbonate (K_2CO_3).

3.1.9 Sodium nitrite ($NaNO_2$).

3.2 Reagent preparation

3.2.1 Food simulants: Prepare according to GB 5009.156.

3.2.2 0.1 mol/L hydrochloric acid solution: Prepare according to GB 5009.156.

3.2.3 0.1 mol/L sodium hydroxide solution: Prepare according to GB 5009.156.

3.2.4 1 mol/L hydrochloric acid solution: Measure 83 mL of hydrochloric acid and slowly add it to 500 mL of water. After the solution cools to room temperature, transfer it to a 1 L volumetric flask. Add water to the mark and mix well.

3.2.5 5 mol/L sodium hydroxide solution: Weigh 5.0 g of sodium hydroxide into a polyethylene or polytetrafluoroethylene plastic beaker. Add 250 mL of water to dissolve.

After cooling, transfer to a plastic (except PET) bottle and set aside.

3.2.6 Artificial saliva: Prepare according to GB 5009.156, or purchase commercially available artificial saliva.

3.3 Standard products/substances

15 N-nitrosamine compound standard substances: Purity is >98%. For detailed information, see Table A.1 in Annex A, or standard substances certified by the state and awarded with standard substance certificates.

3.4 Preparation of standard solution

3.4.1 15 N-nitrosamines mixed standard stock solution (100 mg/L): Accurately weigh 25 mg (accurate to 0.1 mg) of each of the 15 N-nitrosamines standard substances. Dissolve in anhydrous ethanol and transfer to a 250 mL brown volumetric flask. Add ethanol to the mark. Mix well. Transfer the solution to a brown glass container. Store at -18℃ away from light. Shelf life is 6 months.

3.4.2 N-nitrosamines mixed standard intermediate solution (1.00 mg/L): Accurately pipette 1.00 mL of 15 N-nitrosamines mixed standard stock solutions (100 mg/L) into a 100 mL brown volumetric flask. Add anhydrous ethanol to the mark. Mix well. Transfer the solution to a brown glass container. Store at -18℃ away from light. Shelf life 3 months.

3.4.3 Mixed standard series working solution: Pipette 0.50 mL, 1.00 mL, 2.00 mL, 3.00 mL, 4.00 mL, and 5.00 mL of the mixed standard intermediate solution (1.00 mg/L) of N-nitrosamine compounds into six 10 mL brown volumetric flasks. Add anhydrous ethanol to the mark. Mix well. The mass concentrations of the 15 N-nitrosamine compounds are 0.0500 mg/L, 0.100 mg/L, 0.200 mg/L, 0.300 mg/L, 0.400 mg/L, and 0.500 mg/L mixed standard series working solutions. Prepare them before use.

4 Instruments and equipment

4.1 Gas chromatograph-mass spectrometer (GC-MS): equipped with electron impact source (EI source).

4.2 Electronic balance: sensitivity is 0.1 g and 0.1 mg respectively.

4.3 Electric heating blast drying oven.

4.4 Rotary evaporator: negative pressure device and water-cooling equipment.

4.5 Nitrogen blowing instrument.

4.6 pH meter: accuracy is 0.1.

4.7 Microporous filter membrane: nylon; pore size is 0.22 μm.

4.8 Separatory funnel: 150 mL, equipped with polytetrafluoroethylene stopper.

5 Analysis steps

5.1 Migration test

Food contact materials and products shall be subjected to migration tests in accordance with the requirements of GB 31604.1 and GB 5009.156. Rubber consumables and tools, such as sealing rings, gloves, cutting tools, etc., shall be avoided in migration tests. The immersion solution obtained in the migration test shall be tested as soon as possible after returning to room temperature.

5.2 Preparation of test solution

5.2.1 Preparation of N-nitrosamine compound migration test solution

5.2.1.1 Treatment of immersion solution

Add the specified volumes of soaking solution and 5 mol/L sodium hydroxide solution to the separatory funnel in order according to the requirements in Table 1. Mix well. Then add the specified volumes of water and ethanol in Table 1. Mix well.

5.2.1.2 Extraction and concentration of N-nitrosamines

Add 30.0 mL of dichloromethane to the separatory funnel. Shake the funnel slightly counterclockwise. Cover the lid. Vigorously shake for 15 s (release air if necessary). Open the lid. Let stand to separate the layers. Collect the lower layer of extract. Repeat the extraction twice with 30.0 mL of dichloromethane. Combine the extracts. Shake well. Concentrate the extract to about 5 mL by rotary evaporation under reduced pressure at 18℃~25℃ water bath temperature. Blow nitrogen to 0.8 mL~0.9 mL. Transfer to a 1.0 mL brown volumetric flask. Make up to volume with anhydrous ethanol. Filter through a microporous filter membrane and test.

c) Program temperature conditions: initial temperature is 60 ℃. Hold for 2 min. Increase to 82℃ at 15℃/min. Increase to 88℃ at 1℃/min. Increase to 110℃ at 15℃/min and hold for 7 min. Then increase to 240℃ at 15℃/min. Hold for 7 min;

d) Carrier gas: helium (purity ≥ 99.999%);

e) Flow rate: 1.2 mL/min;

f) Injection method: splitless injection;

g) Injection volume: 1 μL.

5.3.2 Mass spectrometry reference conditions

The mass spectrometry reference conditions are as follows:

a) Interface temperature: 250℃;

b) Ion source temperature: 250℃;

c) Ionization mode: electron impact source (EI source);

d) Solvent delay: 5 min;

e) Scan mode: selected ion scan (SIM). The ion parameters are shown in Table B.1 in Annex B.

5.4 Drawing of standard curve

According to the instrument reference conditions listed in 5.3, the mixed standard series working solutions are sampled and measured in sequence. The standard curve is drawn with the mass concentration of N-nitrosamine compounds in the mixed standard series working solutions as the abscissa and the corresponding quantitative ion peak area as the ordinate. The chromatogram of the 15 N-nitrosamine compound standard working solutions is shown in Figure B.1.

5.5 Determination of specimen solution

5.5.1 Qualitative determination

According to the listed instrument reference conditions, measure the specimen solution and the mixed standard series working solution. If the deviation of the mass chromatographic peak retention time of the analyte in the test solution and the standard solution is within the range of ±0.5%, and in the specimen mass spectrum after background subtraction, all qualifier ions appear and the signal-to-noise ratio is ≥3, and the relative abundance of the qualifier ions and the relative abundance deviation of the corresponding substance in the standard working solution with equivalent concentration do not exceed the provisions of Table 3, then it can be judged that the corresponding

analyte exists in the specimen.

5.5.2 Quantitative determination

Inject the test solution and blank test solution into GC-MS. Obtain the peak area of each N-nitrosamine compound. Obtain the concentration of each N-nitrosamine compound in the test solution based on the standard curve. If the content of each N-nitrosamine compound exceeds the range of the standard curve, the test solution can be appropriately diluted and re-measured.

5.5.3 Qualitative confirmation of N-nitrosamine compounds

If the total amount of N-nitrosamine compounds migrated exceeds the limit requirements of the product standard, it shall be confirmed through one of the following ways.

a) Using the property of N-nitrosamine compounds decomposing under ultraviolet radiation, transfer the remaining test solution into a transparent injection bottle. Place it under 365 nm ultraviolet light. After irradiation for 3 h at a distance of about 24 cm from the lamp, re-measure with GC-MS. If the peak area of the chromatographic peak corresponding to the N-nitrosamine compound detected in the test solution after irradiation decreases to less than 60% of the original peak area, it can be confirmed that the detected substance is the corresponding N-nitrosamine compound, otherwise it can be determined that the detected compound is a false positive. This method is not applicable to N-nitroso-N-methylaniline and N-nitroso-N-ethylaniline.

b) Refer to the method in 5.5.3.a) and confirm by gas chromatography-thermal energy analyzer (GC-TEA).

6 Presentation of analysis results

6.1 Calculation of migration of N-nitrosamine compounds

6.1.1 Calculation of specific migration amount of single N-nitrosamine in food contact materials and articles (expressed in mg/kg)

When the specific migration amount of a single N-nitrosamine in food contact materials and articles is expressed in mg/kg, it shall be calculated according to formula (1).

lower than its detection limit, it shall be recorded as "not detected" or "ND". Its value is treated as zero.

7 Precision

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

8 Limit of detection and limit of quantitation

The detection limit of this method for the content of a single N-nitrosamine in 95% (volume fraction) ethanol soaking solution is 1.3 μg/L; the quantification limit is 2.50 μg/L. The detection limit of a single N-nitrosamine in other soaking solutions is 0.7 μg/L; the quantification limit is 1.25 μg/L. In the same soaking solution and under the specified nitrosation conditions, if a single N-nitrosamine that migrates directly is not detected, the detection limit and quantification limit of a single N-nitrosamine product (calculated as the corresponding N-nitrosamine) are the same as the detection limit and quantification limit of the corresponding N-nitrosamine compound content. The detection limit and quantification limit of each N-nitrosamine compound and N-nitrosamine product migration amount are calculated according to Chapter 6.

9 Others

The following precautions shall be observed during the test:

a) After the dichloromethane and anhydrous ethanol used in the analysis are concentrated 60 times, no N-nitrosamine compounds shall be detected (i.e., the signal-to-noise ratio S/N <3);

b) Each standard solution must be prepared in a well-ventilated environment;

c) The mixed standard series working solution shall be treated as required in 5.5.3 for at least 14 h before being discarded. Other high-concentration standard solutions shall be diluted with water to less than 0.5 mg/L and treated as required in 5.5.3 for at least 14 h before being discarded;

d) During the test, contamination caused by rubber items (such as gloves, sealing pads, cutting tools, etc.) shall be avoided.

Method Two -- Gas chromatography-thermal analysis method

10 Principle

The N-nitrosamine compounds in the soaking solution obtained from the migration test are extracted with dichloromethane under alkaline conditions, concentrated, and then determined by gas chromatography-thermal energy analyzer (GC-TEA). The external standard method is used for quantification to obtain the migration amount of N-nitrosamine compounds directly migrated.

The N-nitrosamine-derivable products in the immersion solution obtained in the migration test react with nitrite in artificial saliva under acidic conditions to generate N-nitrosamine compounds. The N-nitrosamine compounds generated by the reaction and the N-nitrosamine compounds directly migrated are simultaneously determined according to the above steps to obtain the sum of the migration amounts of the N-nitrosamine-derivable products and the N-nitrosamine compounds directly migrated. The migration amount of a single N-nitrosamine-derivable product is calculated by subtracting the migration amount of the corresponding N-nitrosamine compound directly migrated from the sum of the measured migration amounts of the two.

11 Reagents and materials

Unless otherwise specified, all reagents used in this method are analytically pure and water is Grade One water as specified in GB/T 6682.

11.1 Reagents

Same as 3.1.

11.2 Reagent preparation

Same as 3.2.

11.3 Standard products/substances

Same as 3.3.

11.4 Preparation of standard solution

11.4.1 Mixed standard stock solution of 15 N-nitrosamine compounds (100 mg/L): Same as 3.4.1.

11.4.2 N-nitrosamines mixed standard intermediate solution (1.00 mg/L): Same as 3.4.2.

11.4.3 Mixed standard series working solution: Pipette 0.25 mL, 0.50 mL, 1.00 mL, 2.50 mL, and 5.00 mL of the mixed standard intermediate solution (1.00 mg/L) of N-nitrosamine compounds into five 10 mL brown volumetric flasks. Add anhydrous ethanol to the mark. Mix well. The mixed standard working solutions of 15 N-

nitrosamine compounds with mass concentrations of 0.0250 mg/L, 0.0500 mg/L, 0.100 mg/L, 0.250 mg/L, and 0.500 mg/L are obtained. Prepare before use.

12 Instruments and equipment

12.1 Gas chromatography-thermal energy analyzer (GC-TEA).

12.2 Others are the same as 4.2~4.7.

13 Analysis steps

13.1 Migration test

Same as 5.1.

13.2 Preparation of test solution

Same as 5.2.

13.3 Instrument reference conditions

13.3.1 Gas chromatography conditions

The gas chromatography reference conditions are as follows:

a) Chromatographic column: modified polyethylene glycol chromatographic column, 30 m × 0.25 mm (inner diameter) × 0.32 μm (film thickness) or equivalent performance;

b) Inlet temperature: 170℃;

c) Program temperature conditions: Initial temperature is 60℃. Keep for 2 min. Increase to 82℃ at 15 ℃/min. Increase to 88℃ at 1℃/min. Increase to 140℃ at 15℃/min. Keep for 7 min. Increase to 240℃ at 15℃/min. Keep for 7 min;

d) Carrier gas: nitrogen (purity ≥ 99.999%);

e) Flow rate: 1.2 mL/min;

f) Injection method: splitless injection;

g) Injection volume: 2 μL.

13.3.2 Thermal analyzer conditions

The reference conditions of the thermal analyzer are as follows:

a) Interface temperature: 250℃;

b) Pyrolysis chamber temperature: 500℃;

c) Vacuum degree: 59.8 Pa~66.5 Pa;

d) Oxygen pressure: 13.79 kPa;

e) Ozone level: 244.

13.4 Drawing of standard curve

According to the instrument reference conditions listed in 13.3, the mixed standard series working solutions are sampled and measured in sequence. The standard curve is drawn with the mass concentration of N-nitrosamine compounds in the mixed standard series working solutions as the abscissa and the corresponding peak area as the ordinate. The chromatogram of the 15 N-nitrosamine compound standard working solutions is shown in Figure C.1 in Annex C.

13.5 Determination of specimen solution

13.5.1 Qualitative determination

According to the instrument reference conditions listed in 13.3, measure the specimen solution and the mixed standard series working solution. If the deviation of the retention time of the chromatographic peak of the analyte in the test solution and the standard solution is within the range of ±0.5%, it can be judged that the corresponding analyte exists in the specimen.

13.5.2 Quantitative determination

Inject the test solution and blank test solution into GC-TEA to obtain the peak area of each N-nitrosamine compound. According to the standard curve, the concentration of each N-nitrosamine compound in the test solution is obtained. If the content of each N-nitrosamine compound exceeds the range of the standard curve, the test solution can be appropriately diluted and re-measured.

13.5.3 Qualitative confirmation of N-nitrosamine compounds

Same as 5.5.3b).

14 Expression of analytical results

Same as Chapter 6.

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