NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Determination of nonylphenol in foods

食品安全国家标准 食品中壬基酚的测定

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1 Scope and principle

1 The document lays down a liquid chromatography tandem mass spectrometry method for 4-nonylphenol in foods and applies to the determination of 4-nonylphenol in foods.

2 The 4-nonylphenol in the test portion is extracted with acetonitrile or with an acetonitrile solution, separated by high performance liquid chromatography, detected by a tandem mass spectrometer and quantified by the isotope internal standard method.

3 Reagents and materials

3 Unless stated otherwise the reagents are of chromatographic grade and the water is grade 1 water as specified in GB/T 6682.

3.1 The reagents are methanol, acetonitrile and ammonia solution of concentration not less than 25 %.

3.2 Two solutions are prepared: a 70 % acetonitrile solution, made by measuring 700 mL of acetonitrile and diluting to 1 000 mL with water, and a 0.1 % ammonia solution, made by taking 1 mL of ammonia solution and diluting to 1 000 mL with water.

3.3 The standards are 4-nonylphenol, formula C15H24O, CAS number 84852-15-3, of purity not less than 99 % or certified as a national reference material, and the isotope internal standard 3,6,3-nonylphenol labelled with carbon 13 at six positions, CAS number 1173020-38-6, also of purity not less than 99 %.

3.4 The 4-nonylphenol stock standard solution at 100 mg/L is prepared by weighing 10 mg

of the standard to 0.000 01 g into a 10 mL small beaker, dissolving in methanol, transferring to a 100 mL brown volumetric flask, making up to the mark with methanol and mixing; it keeps for 6 months at -18 °C. The isotope internal standard stock solution at 100 mg/L is prepared in exactly the same way and keeps equally for 6 months at -18 °C.

3.4 The 4-nonylphenol intermediate standard solution at 1.0 mg/L is prepared by pipetting 1.0 mL of the stock solution at 100 mg/L into a 100 mL brown volumetric flask, making up to the mark with methanol and mixing; it keeps for 3 months at -18 °C. The isotope internal standard working solution at 1.0 mg/L is prepared in the same way and keeps for 3 months at -18 °C.

3.4.5 The series of 4-nonylphenol working standard solutions is prepared by pipetting 2.50 mL of the intermediate solution at 1.0 mg/L into a 25 mL brown volumetric flask, diluting to the mark with methanol and shaking; then 100 µL, 250 µL, 500 µL, 1.25 mL, 2.50 mL and 5.00 mL of that dilution, together with 125 µL of the internal standard intermediate solution at 1.0 mg/L, are transferred into 25 mL brown volumetric flasks and made up to the mark with acetonitrile and mixed. The 4-nonylphenol mass concentrations of the series are, in order, 0.40 ng/mL, 1.00 ng/mL, 2.00 ng/mL, 5.00 ng/mL, 10.0 ng/mL and 20.0 ng/mL, and the mass concentration of the internal standard in each working solution is 5.0 ng/mL. The series is prepared fresh before use.

3.5 The material used is polytetrafluoroethylene centrifuge tubes of 5 mL and 10 mL.

4 Instruments, equipment and preparation of the test sample

4 The instruments are a liquid chromatograph tandem mass spectrometer fitted with an electrospray ion source, an analytical balance with readabilities of 0.000 01 g and 0.01 g, a vortex mixer, a high speed refrigerated centrifuge working at 4 °C with a speed of at least 10 000 r/min, a homogeniser, a mill and an ultrasonic cleaner.

5 Liquid test samples are shaken up, semi-solid and powdered samples of even matrix are shaken up, and other samples are homogenised or ground until even.

6 Analytical procedure

6.1.1 For liquid or semi-solid foods, 1 g of the test sample is weighed to 0.01 g into a 5 mL polytetrafluoroethylene centrifuge tube, 25.0 µL of the internal standard working solution at 1.0 mg/L is added and the tube vortexed for 10 s; 3.0 mL of acetonitrile is added, the tube is stoppered and vortexed for 5 s, extracted under ultrasound for 10 min and centrifuged at 10 000 r/min for 5 min at 4 °C; the whole of the upper layer is drawn off into a 5 mL volumetric flask, made up to the mark with acetonitrile and mixed. If the solution is still cloudy it may be centrifuged once more, and the supernatant is measured by liquid chromatography tandem mass spectrometry.

6.1.2 For vegetable oils and fats and for butter, 1 g of the test sample is weighed to 0.01 g

into a 10 mL polytetrafluoroethylene centrifuge tube, butter samples being first warmed in a water bath at 60 °C for about 5 min with occasional gentle shaking to melt the fat; 25.0 µL of the internal standard working solution is added and the tube vortexed for 10 s; 4.0 mL of acetonitrile is added, the tube is vortexed for 2 min and left to stand for 30 min, and after the layers have separated it is centrifuged at 10 000 r/min for 5 min at 4 °C. The whole of the upper layer is transferred to a 5 mL volumetric flask, made up to the mark with acetonitrile and mixed, with a second centrifugation if the solution is still cloudy.

6.1.3 For solid samples, 1 g of the test sample is weighed to 0.01 g into a 10 mL polytetrafluoroethylene centrifuge tube, then 25.0 µL of the internal standard working solution and 5.0 mL of the 70 % acetonitrile solution are added in turn, meat and aquatic samples receiving 5.0 mL of acetonitrile instead; the tube is vortexed for 30 s, stoppered, extracted under ultrasound for 30 min, cooled to room temperature and centrifuged at 10 000 r/min for 5 min at 4 °C, and the upper layer is treated as above.

6.1.4 For milk powder and infant formula, 1 g of the test sample is weighed to 0.01 g into a 5 mL graduated glass tube, then 25.0 µL of the internal standard working solution and about 1.5 mL of hot water at around 50 °C are added in turn; the tube is vortexed for 10 s until dissolution is complete and extracted under ultrasound for 10 min; 3.5 mL of acetonitrile is added, the tube is stoppered and shaken vigorously for 5 s, the whole of the upper layer is transferred to a 5 mL polytetrafluoroethylene centrifuge tube and centrifuged at 10 000 r/min for 5 min at 4 °C, and the upper layer is treated as above.

6.2.1 The reference liquid chromatographic conditions are a C18 column of 4.6 mm x 50 mm and 1.8 µm with a guard column of 4.6 mm x 5 mm and 1.8 µm, or columns of equivalent performance; a mobile phase of 0.1 % ammonia solution and methanol in the volume ratio 10 to 90; a flow rate of 0.35 mL/min; a column temperature of 30 °C; and an injection volume of 5 µL.

6.2.2 The reference mass spectrometric conditions are electrospray ionisation in negative ion mode; multiple reaction monitoring; a drying gas temperature of 340 °C with nitrogen; a drying gas flow of 8 L/min of nitrogen; a nebuliser pressure of 40 psi, with a note that 1 psi equals 0.006 894 8 MPa; and a capillary voltage of 4 000 V. Table 1 gives the main mass spectrometric parameters. For 4-nonylphenol the precursor ion is 219.2, the product ions are 133.0, marked as the quantifier, and 147.0, the fragmentor voltage is 120 V and the collision energies are 30 eV and 25 eV. For the 4-nonylphenol isotope internal standard the precursor ion is 225.2, the product ion is 139.1, the fragmentor voltage is 100 V and the collision energy is 30 eV. A note adds that the parameters may differ between instruments and are to be optimised before measurement until they meet the requirements of the method.

6.3 For the calibration curve the series of working standard solutions is injected in turn and the peak areas are measured. The curve is drawn with the ratio of the concentration of 4-nonylphenol to the concentration of the internal standard in the series on the horizontal

axis and the ratio of the peak area of 4-nonylphenol to that of the internal standard on the vertical axis. The multiple reaction monitoring chromatogram of the working standard solution is shown in Annex A.

6.4.1 For identification, the retention time of the analyte in the sample has to fall within +/- 2.5 % of that in the standard solution under the same conditions, and the relative abundance of the qualifier ion in the sample, compared with that in a standard solution of similar concentration, has to stay within the tolerance of Table 2. Table 2 gives the maximum permitted deviation of the relative ion abundance: for an abundance above 50 %, +/- 20 %; above 20 % and up to 50 %, +/- 25 %; above 10 % and up to 20 %, +/- 30 %; not above 10 %, +/- 50 %.

6.4.2 to 6.6 For quantification the sample solution is injected, the ratio of the peak area of 4-nonylphenol to that of the internal standard is obtained and the concentration in the test solution is read from the calibration curve. A blank test is run through the whole procedure without a test portion. All reagents and vessels used are subjected to a blank test before use; if the blank exceeds one times the detection limit, the reagents are redistilled or the reagents and vessels replaced so that the blank stays below one times the detection limit.

7 Expression of the result, precision and other

7 The 4-nonylphenol content of the test sample is computed from formula 1, whose symbols are the content of 4-nonylphenol in the test sample in micrograms per kilogram, the mass concentration of 4-nonylphenol in the test solution read from the calibration curve in nanograms per millilitre, the mass concentration of 4-nonylphenol in the blank test solution read from the same curve in nanograms per millilitre, the volume to which the test portion was made up in millilitres, the mass of the test portion in grams and a conversion factor of 1 000. The result is the arithmetic mean of two independent determinations obtained under repeatability conditions and is reported to three significant figures.

8 Under repeatability conditions the absolute difference between two independent results must not exceed 15 % of their arithmetic mean.

9 With a test portion of 1 g and a made-up volume of 5 mL, the detection limit is 2.0 µg/kg and the limit of quantification is 5.0 µg/kg.

Annex A carries the multiple reaction monitoring chromatogram of the 4-nonylphenol standard at 10 µg/L and of its internal standard, as figure A.1.