GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.284-2021

National Food Safety Standard - Determination of vanillin, methyl vanillin, ethyl vanillin and coumarin in foods

Issued on: September 07, 2021 **Implemented on: March 07, 2022**

Issued by: National Health Commission of the People's Republic of China; State Administration for Market Regulation.

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National Food Safety Standard - Determination of vanillin, methyl vanillin, ethyl vanillin and coumarin in foods

1 Scope

This Standard specifies the determination methods of vanillin, methyl vanillin, ethyl vanillin and coumarin in foods.

This Standard applies to the determination of vanillin, methyl vanillin, ethyl vanillin and coumarin in infant formula, complementary foods for infants and young children, cakes, candies, milk and dairy products, beverages, and wheat flour.

Method I – Liquid chromatography

2 Principle

Add water to mix the sample; then, add acetonitrile for ultrasonic extraction; use liquid chromatography for separation, ultraviolet detector or diode array detector for detection, and external standard method for quantitation.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water as specified in GB/T 6682.

3.1 Reagents and materials

3.1.1 Acetonitrile (CH_3CH): chromatographic pure.

3.1.2 Formic acid (HCOOH): chromatographic pure.

3.1.3 Methanol (CH_3OH): chromatographic pure.

3.1.4 Sodium chloride (NaCl)

3.1.5 Concentrated hydrochloric acid (HCl): 12 mol/L.

3.1.6 Microporous membrane: 0.45 μm, organic phase type.

3.4.3 Standard mixed series working solution: respectively draw 0.2 mL, 1.0 mL and 5.0 mL of standard mixed intermediate solution (10 mg/L); put them in the 10 mL volumetric flasks; besides, respectively draw 0.2 mL, 0.5 mL and 1 mL of standard stock solutions (1 000 mg/L) of vanillin, methyl vanillin, ethyl vanillin and coumarin; put them in the 10 mL volumetric flasks; add methanol-aqueous solution to fix volume to the mark; mix well. The concentrations of standard mixed series working solutions are 0.2 mg/L, 1.0 mg/L, 5.0 mg/L, 20.0 mg/L, 50.0 mg/L, 100.0 mg/L, respectively; prepare them for immediate use.

4 Instruments and apparatuses

4.1 Liquid chromatograph: equipped with diode array or UV detector.

4.2 Balance: the sensitivity is 0.1 mg and 0.01 g.

4.3 Vortex mixer.

4.4 Centrifuge.

4.5 Nitrogen concentrator.

4.6 Ultrasonic generator.

5 Analysis steps

5.1 Sample pretreatment

5.1.1 Sample preparation

Shake liquid samples well; semi-solid samples and powder samples with uniform base materials shall be directly used for the sample extraction in 5.1.2; other samples need to be homogenized or pulverized uniformly.

5.1.2 Sample extraction

5.1.2.1 Infant formula, complementary foods for infants and young children

Weigh 1.00 g of sample; add 10 mL of water and 480 μL of hydrochloric acid solution; vortex for 1 min; add 20 mL of acetonitrile; vortex for 1 min; after 30 min of ultrasonic extraction, add 5 g of sodium chloride; vortex for 2 min; centrifuge at 8 000 r/min for 5 min; take the supernatant to a glass test tube; use nitrogen at 40°C to blow it to near dryness; accurately add 1.0 mL of methanol-aqueous solution to dissolve the residue; pass it through a 0.45 μm microporous membrane; wait for later test.

5.1.2.2 Cakes, candies, milk and dairy products, beverages

Weigh 1.00 g of sample; add 5 mL of water (for gum-based candy, add 10 mL of water, and dissolve in a water bath at 50 °C); vortex for 1 min; add 20 mL of acetonitrile; vortex for 1 min; after 30 min of ultrasonic extraction, add 5 g of sodium chloride; vortex for 2 min; centrifuge at 8 000 r/min for 5 min (for cake samples with high fat content, take frozen centrifugation at 8 000 r/min); take the supernatant to a glass test tube; use nitrogen to blow at 40 °C to nearly dry; accurately add 1.0 mL of methanol-aqueous solution to dissolve the residue; pass it through a 0.45 μm microporous membrane for later test.

5.1.2.3 Wheat flour

Weigh 1.00 g of sample; add 10 mL of water and 240 μL of hydrochloric acid solution; vortex for 1 min; add 20 mL of acetonitrile; vortex for 1 min; after 30 min of ultrasonic extraction, add 5 g of sodium chloride; vortex for 2 min; centrifuge at 8 000 r/min for 5 min; take the supernatant to a glass test tube; use nitrogen at 40°C to blow it to near dryness; accurately add 1.0 mL of methanol-aqueous solution to dissolve the residue; pass it through a 0.45 μm microporous membrane; wait for later test.

5.2 Apparatus reference conditions

5.2.1 Chromatographic column: C_{18} column, 250 mm × 4.6 mm (inner diameter), 5 μm (filler particle size) or equivalent.

5.2.2 Column temperature: 30 °C.

5.2.3 Injection volume: 10 μL;

5.2.4 Detection wavelength: 279 nm.

5.2.5 Flow velocity: 1.0 mL/min.

5.2.6 Mobile phase

Phase A: 0.5% formic acid solution;

Phase B: Acetonitrile.

Gradient elution is shown in Table 1.

Table 1 – Gradient elution procedure

polymer which is composed of two monomers, lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidone, in a certain proportion. Use 3 mL of methanol and 3 mL of water to activate it before use.

10.1.7 Microporous membrane: 0.22 μm, organic phase type.

10.2 Preparation of reagents

10.2.1 0.1% formic acid methanol solution: draw 1 mL of formic acid and dissolve it in methanol and dilute to 1 000 mL; prepare it for immediate use.

10.2.2 Hydrochloric acid solution (1 mol/L): draw 8.33 mL of concentrated hydrochloric acid; dissolve it in water and dilute to 100 mL; prepare it for immediate use.

10.2.3 Methanol-aqueous solution (4+1): mix methanol and water at 4:1 (volume ratio) uniformly.

10.2.4 0.5% formic acid solution: draw 5 mL of formic acid; dissolve it in water and dilute to 1 000 mL; prepare it for immediate use.

10.3 Standard substances

10.3.1 Vanillin standard substance ($C_8H_8O_3$, CAS number: 121-33-5): purity ≥98%, or a standard substance that is certified by the nation and granted a standard substance certificate.

10.3.2 Methyl vanillin standard substance ($C_9H_{10}O_3$, CAS number: 120-14-9): purity ≥98%, or a standard substance that is certified by the nation and granted a standard substance certificate.

10.3.3 Ethyl vanillin standard substance ($C_9H_{10}O_3$, CAS number: 121-32-4): purity ≥98%, or a standard substance that is certified by the nation and granted a standard substance certificate.

10.3.4 Coumarin standard substance ($C_9H_6O_2$, CAS number: 91-64-5): purity ≥97%, or a standard substance that is certified by the nation and granted a standard substance certificate.

10.3.5 D_3-vanillin standard substance ($C_8H_5D_3O_3$, CAS number: 74495-74-2): purity ≥98%.

10.3.6 D_3-methyl vanillin standard substance ($C_9H_7D_3O_3$, CAS number: 143318-06-3): purity ≥98%.

10.3.7 D_5-ethyl vanillin standard substance ($C_9H_5D_5O_3$, CAS number: 1335401-74-5): purity ≥98%.

10.3.8 D_4-coumarin standard substance ($C_9H_2D_4O_2$, CAS number: 185056-83-1): purity ≥98%.

10.4 Preparation of standard solutions

10.4.1 Standard stock solution (1 000 mg/L): same as 3.4.1.

10.4.2 Standard mixed intermediate solution (10 mg/L): same as 3.4.2.

10.4.3 Internal standard stock solution (1 000 mg/L): accurately weigh 10 mg of D_3-vanillin, D_3-methyl vanillin, D_5-ethyl vanillin and D_4-coumarin (accurate to 0.1 mg) respectively; place them in the 10 mL volumetric flasks respectively; use 0.1% formic acid methanol solution to dissolve and dilute to the mark; mix well; transfer the solution to a brown glass container; store at -18 °C in the dark for 8 months.

10.4.4 Internal standard mixed intermediate solution (10 mg/L): draw 1.00 mL of the standard stock solutions of D_3-vanillin, D_3-methyl vanillin, D_5-ethyl vanillin and D_4-coumarin (1 000 mg/L) respectively; place them in the 100 mL volumetric flasks; add 0.1% formic acid methanol solution to fix volume to the mark; mix well. Transfer the solution to a brown glass container; store at -18 °C in the dark for 3 months.

10.4.5 Standard and internal standard mixed series working solutions: respectively draw 0.05 mL, 0.2 mL, 0.5 mL, 1 mL, 2 mL of the standard mixed intermediate solution (10 mg/L) and 0.1 mL of the internal standard mixed intermediate solution (10 mg/L); place them to in the 10 mL volumetric flasks; add methanol-aqueous solution to the mark; mix well. The concentrations of the standard and internal standard mixed series working solutions are 0.05 mg/L, 0.2 mg/L, 0.5 mg/L, 1.0 mg/L and 2.0 mg/L respectively; the concentration of the internal standard is 0.1 mg/L. Prepare when necessary.

11 Instruments and apparatuses

11.1 Liquid chromatography tandem quadrupole mass spectrometer: equipped with electrospray ionization source.

11.2 Balance: the sensitivity is 0.1 mg and 0.01 g.

11.3 Vortex mixer.

11.4 Centrifuge.

11.5 High-speed refrigerated centrifuge.

11.6 Nitrogen concentrator.

14 Precision

The absolute difference of two independent test results under repeatability cannot exceed 15% of the arithmetic mean value.

15 Others

The detection-limit of this method is 0.02 mg/kg; the quantitation-limit is 0.05 mg/kg.

Method III – Gas chromatography-mass spectrometry

16 Principle

Add water to mix the sample well; then, use acetonitrile for ultrasonic extraction; use the solid-phase extraction column to purify the extracting solution; then, use gas chromatograph-mass spectrometer for determination, and internal standard method for quantitation.

17 Reagents and materials

17.1 Reagents and materials

Same as 10.1.

17.2 Preparation of reagents

Same as 10.2.

17.3 Standard substances

Same as 10.3.

17.4 Preparation of standard solutions

17.4.1 Standard stock solution (1 000 mg/L): same as 3.4.1.

17.4.2 Standard mixed intermediate solution (10 mg/L): same as 3.4.2.

17.4.3 Internal standard stock solution (1 000 mg/L): same as 10.4.3.

17.4.4 Internal standard mixed intermediate solution (10 mg/L): same as 10.4.4.

17.4.5 Standard and internal standard mixed series working solution: same as 10.4.5.

18 Instruments and apparatuses

18.1 Gas chromatograph-mass spectrometer: with electron bombardment ionization source.

18.2 Balance: the sensitivity is 0.1mg and 0.01g.

18.3 Vortex mixer.

18.4 Centrifuge.

18.5 High-speed refrigerated centrifuge.

18.6 Nitrogen concentrator.

18.7 Ultrasonic generator.

18.8 Solid-phase extraction device.

19 Analysis steps

19.1 Sample pretreatment

19.1.1 Sample preparation

Same as 12.1.1.

19.1.2 Sample extraction

Same as 12.1.2.

19.1.3 Sample purification

Use nitrogen to blow the supernatant that is obtained in 19.1.2 to near dryness at 40 °C; add 1 mL of methanol to dissolve the residue; use water to fix volume to 10 mL, so that the sample solution passes through the solid-phase extraction column at a flow rate of less than 1 mL/min. After all the sample solution flows out, use 5 mL of water to rinse; vacuum dry at negative pressure; use 10 mL of methanol-aqueous solution to elute; vacuum dry at negative pressure; collect the eluate; use nitrogen to blow at 40 °C to near dryness; accurately add 1 mL of methanol to dissolve the residue; after passing it through a 0.22 μm microporous membrane, test it.

19.2 Apparatus reference conditions

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