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 7, 2021 **Implemented on: March 7, 2022**

Issued by: National Health Commission;
State Administration for Market Regulation

Table of Contents

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.

3.2 Preparation of reagents

3.2.1 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.

3.2.2 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.

3.3.1 Vanillin standard substance (C8H8O3, CAS number. 121-33-5). purity

>=98%, or a standard substance that is certified by the nation and granted a standard substance certificate.

3.4.1 Standard stock solution (1 000 mg/L). accurately weigh 10 mg (accurate

to 0.1 mg) of vanillin, methyl vanillin, ethyl vanillin and coumarin; 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.

3.4.2 Standard mixed intermediate solution (10 mg/L). respectively draw 1.00

mL of standard stock solutions (1 000 mg/L) of vanillin, methyl vanillin, ethyl vanillin and coumarin; place them in the 100 mL volumetric flasks; add 0.1% methanol formic acid 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.

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.

5.1.2.1 Infant formula, complementary foods for infants and young children

Weigh 1.00 g of sample; add 10 mL of water and 480 uL of hydrochloric acid solution; vortex for 1 min;

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;

5.2 Apparatus reference conditions

5.2.1 Chromatographic column. C18 column, 250 mm x 4.6 mm (inner diameter), 5 um (filler particle size) or equivalent.

5.2.2 Column temperature. 30 °C.

5.2.3 Injection volume. 10 uL;

5.3 Preparation of the standard curve

Inject the standard mixed series working solutions into the liquid chromatograph respectively; measure the corresponding peak areas. Take the concentrations of vanillin, methyl vanillin, ethyl vanillin and coumarin in the standard series working solutions as the abscissa, and the peak area as the ordinate, to draw a standard curve. For the chromatograms of standard solutions of vanillin, methyl vanillin, ethyl vanillin and coumarin, see Figure A.1 in Appendix A.

6 Expression of the analysis results

Calculate the content of the tested compound in the sample according to Formula (1).

7 Precision

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

8 Others

The detection-limit of this method is 0.07 mg/kg; the quantitation-limit is 0.20 mg/kg.

9 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 liquid chromatograph-mass spectrometer/mass spectrometer for determination, and internal standard method for quantitation.

10 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.

10.1.6 Polar group-containing reverse-phase polymer solid-phase extraction

column.

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.

10.3.1 Vanillin standard substance (C8H8O3, CAS number. 121-33-5). purity

>=98%, or a standard substance that is certified by the nation and granted a standard substance certificate.

10.3.6 D3-methyl vanillin standard substance (C9H7D3O3, CAS number.

143318-06-3). purity >=98%.

10.4.4 Internal standard mixed intermediate solution (10 mg/L). draw 1.00 mL

of the standard stock solutions of D3-vanillin, D3-methyl vanillin, D5-ethyl vanillin and D4-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